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# Novel bioglasses for bone tissue repair and regeneration: Effect of glass design on sintering ability, ion release and biocompatibility

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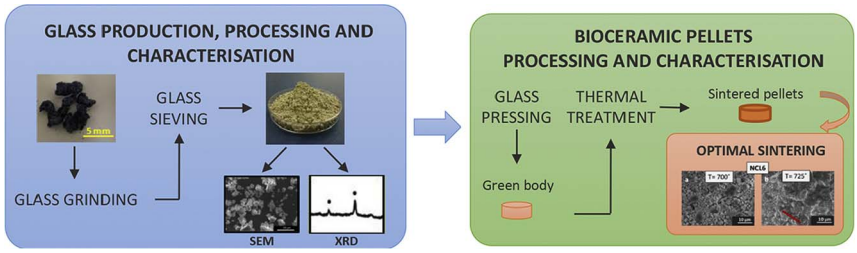
ABSTRACT

Eight novel silicate, phosphate and borate glass compositions (coded as NCLx, where x = 1 to 8), containing different oxides (*i.e.* MgO, $MnO_2$, $Al_2O_3$, $CaF_2$, $Fe_2O_3$, ZnO, CuO, $Cr_2O_3$) were designed and evaluated alongside apatite-wollastonite (used as comparison material), as potential biomaterials for bone tissue repair and regeneration. Glass frits of all the formulations were processed to have particle sizes under 53 μm, with their morphology and dimensions subsequently investigated by scanning electron microscopy (SEM). In order to establish the nature of the raw glass powders, X-ray diffraction (XRD) analysis was also performed. The sintering ability of the novel materials was determined by using hot stage microscopy (HSM). Ionic release potential was assessed by inductively coupled plasma optical emission spectroscopy (ICP-OES). Finally, the cytotoxic effect of the novel glass powders was evaluated for different glass concentrations *via* a colorimetric assay, on which basis three formulations are considered promising biomaterials.

GRAPHICAL ABSTRACT




## 1. Introduction

The first reported use of a glass intended for bone tissue repair dates back to 1969, when Professor L. Hench proposed a composition in the $Na_2O$-CaO-$SiO_2$-$P_2O_5$ system, designated as bioglass 45S5 [1,2], commercially known as Bioglass®.

Although Bioglass® proved to be an excellent material, considered for long time the gold standard bone substitute, it suffers from several drawbacks. Specifically, the main difficulties are related to the material processing in form of 3D porous scaffolds, due to the limited ability of this glass in sintering [3]. Additionally, other weaknesses include: its slow degradation kinetic with the consequent difficulties to match the formation rate of new tissue, and the abrupt pH variations of the biological microenvironment, due to the increase in the concentration of ions such as $Na^+$ and $Ca^{2+}$, especially in the short term when the release is faster [4–6].

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**Table 1**
Rationale of the novel glass compositions.

| CODE | MAIN NETWORK FORMER | AIM |
|---|---|---|
| NCL1 | $SiO_2$ | To develop a material with osteogenic properties, mainly determined by the presence of a high amount of silica. |
| NCL2 | $SiO_2$ | To develop a load-bearing material with osteogenic properties and tailored degradation rate. |
| NCL3 | $B_2O_3$ | To develop a material with improved degradation rate and appropriate level of bioactivity as well as mechanical properties |
| NCL4 | $B_2O_3$ | To develop a material with tailored degradation rate and osteogenic effects. |
| NCL5 | $P_2O_5$ | To develop a resorbable glass with controlled degradation rate. |
| NCL6 | $P_2O_5$ | To develop a resorbable glass with controlled degradation rate, and improved mechanical strength |
| NCL7 | $SiO_2$ | To develop a material with antibacterial properties, mainly determined by the presence of silver oxide, and a good level of bioactivity. |
| NCL8 | $SiO_2$ | To develop a material with osteogenic properties and tailored degradation rate for non-load bearing applications. |

Worldwide many researchers have used the $SiO_2$-$Na_2O$-CaO-$P_2O_5$ system as a template for developing new silica-based compositions [7]. Subsequently, many formulations in the phosphate and borate-based system have been also designed to overcome the Bioglass® and silicate-based glass limitations [8–11], and thus to meet the set of requirements that are both crucial and necessary for optimised tissue-engineered substitutes [12].

The possibility to tailor glass properties by doping the main composition with network modifiers and/or intermediate oxides [13–20] offers significant potential for this class of biomaterials. In addition to promoting bone bonding, the release of soluble ions (*i.e.* Si, Ca, P and Na) from these glasses have been demonstrated to promote cell proliferation, differentiation and activate gene expression [20–24]. Furthermore, it has been also revealed that even slight changes in the glass main formulation can substantially affect the material behaviour, particularly the physico-chemical and mechanical properties, dissolution rate, bioactivity and bioresorbability [5,16,18,25–27].

However, there are still several criticisms related to the clinical use of this class of biomaterials in bone repair [12]. Firstly, whether or not glass dissolution products have a positive effect on adult stem cells is still an open debate [28]. Secondly, they have often proved inadequate when used in load-bearing bone defects, due to their low tensile strength and fracture toughness [29]. Ultimately, there are no large-scale porous bioactive glasses on the market, thus their commercial success as bone scaffolds is limited [6,12].

The aim of this work was the development and characterisation of eight novel silicate, phosphate and borate glass formulations (coded as NCLx, where x = 1 to 8), containing different oxides and in diverse molar percentages as promising biomaterials for the repair and regeneration of bone tissue.

## 2. Materials and methods

### 2.1. Development of novel glass formulations

Based on the current state of the art, the eight bioceramic formulations were developed using: silicon dioxide, phosphorous pentoxide and boron trioxide as network formers due to their promising bioactive potential [1,30,31], distinctive resorbable properties [32,33], and customable degradation rate [5,34], along with a range of different doping agents (*i.e.* MgO, $MnO_2$, $Al_2O_3$, $CaF_2$, $Fe_2O_3$, ZnO, CuO, $Cr_2O_3$), which were used to tailor the properties of the main composition [35–41]. The rationale and innovative characteristics of the novel materials are reported in Table 1. Additionally, considering the excellent biocompatibility either *in vitro* and *in vivo* of apatite wollastonite (AW) [42–44], and the fact that it has been adopted for a broad range of medical applications, either in the form of powder, porous structures or bulk material [45,46], AW glass-ceramic was used as comparison material in this study.

### 2.2. Glass production and processing

The novel glasses were produced and supplied by Glass Technology Service (GTS) Ltd. (Sheffield, UK) along with AW. Briefly, the individual components (see Table 2) of each formulation were weighed out and then mixed together to obtain a uniform blend, which was subsequently melted in platinum crucibles at temperatures up to 1500 °C. The individual melts of glass were cast as solid blocks and then thermally shocked in de-ionised water to produce the precursor materials, known as frits.

Glass frits of all the compositions were ground in a one-bowl zirconia ball milling machine (Planetary Mono Mill Pulverisette 6, Fritsch GmbH, Germany) using a rotational speed of 400 rpm for 30 min, and then sieved using a mechanical sieve shaker (Impact Test Equipment Ltd., UK) to have a final particle size about 20 μm and below 53 μm.

Powders were prepared for pressing through mixing with an isopropanol solution (Sigma Aldrich, UK) in the proportion 1:3 (w/w). Powders were then pressed using an automatic hydraulic press (Specac-Atlas™ 8T, Specac Ltd., UK) to make 10 mm diameter and 2.5 mm high pellets. The pressed pellets were then sintered in a furnace (Carbolite 1200 CWF, Carbolite GmbH, Germany), with the sintering times and temperatures defined by the results of the hot stage microscopy analysis, reported in Section 2.3.2.

### 2.3. Physico-chemical characterisation

#### 2.3.1. Microstructural characterisation

Powder glasses and dense pellets were sputtered with a thin layer of gold (approximately 10 nm, sputter time 40 s at 40 mA), and afterward analysed using a Philips XL30 Field-Emission Environmental Scanning Electron Microscope (ESEM FEG), which is fitted with a Rontec Quantax system for the Energy-Dispersive Spectroscopy (EDS) analysis. All the images were taken at an operation voltage of 20 kV, and working distance between 5 and 10 mm.

#### 2.3.2. Hot stage microscopy (HSM)

The sintering ability of the novel glass powders was determined using hot stage microscopy (Misura®, Expert System Solutions, Italy). Tests were performed in air using a heating rate of 10 °C/min up to 1200 °C. Glass powders were manually pressed into a small cylindrical die (2 × 3 mm) and placed on a 10 × 15 × 1 mm alumina support. During the process the specimens were observed by a video camera and images of the changing sample profile were acquired up to 1450 °C. Afterwards, the sample shrinkage at different temperatures was calculated from the variation of the sample area, using the following formula:

$$\text{shrinkage } (\%) = \frac{A_T}{A_0} \times 100$$

where $A_0$ ($mm^2$) was the initial area of the specimen at room temperature and $A_T$ ($mm^2$) was the area of the specimen at the temperature $T$.

#### 2.3.3. XRD Analysis

To investigate the nature of the novel materials. XRD analysis was