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Abstract

In this work, a new bioresorbable phosphate glass (I-CEL2) was prepared in order to use it for the production of 3D-bioresorbable scaffold for bone regeneration. I-CEL2 was characterized to assess its thermal characteristics as well as its bioresorption rate in different medium such as distilled water, Tris-HCl and Simulated Body Fluid (SBF). 3D-macroporous scaffolds were prepared by mixing and pressing I-CEL2 powders and an organic phase and by treating the compact of powders at 550°C for 3 hours. The obtained scaffolds showed a very high porosity and a high resorption rate and are thus suitable candidates for a scaffold to be used as a temporary guide for bone regeneration. The initial response of human marrow stromal cells (hMSCs) has been tested on I-CEL2 surface to describe its biological potential.

Introduction

Synthetic grafts are interesting and challenging candidates for guided bone regeneration. Their 3D porous structure allows a fast vascularisation of the graft and supports the newly formed bone during its regeneration. An ideal scaffolding biomaterial should resorpt with a kinetics matching that of bone regeneration in order to act as a template during bone growth. Phosphate-based glasses are known to be potentially bioresorbable with different solubility rates depending on their composition [1,2]. Their degradation rate is usually linear with time and can be tailored by a careful control of the glass composition. For this reason, phosphate-based glasses can be proposed to produce 3D-macroporous scaffolds that will resorpt *in vivo* with a tailored rate. In this work, a new phosphate-based glass was prepared adding also 3% mol. of SiO_2 due to the ability of silica of stimulating gene expression in osteoblasts and increasing calcium nodules formation as reported in literature [3].

Materials and Methods

In this work a new phosphate glass belonging to the system P_2O_5-SiO_2-CaO-MgO-Na_2O-K_2O (I-CEL2) was prepared by melting the raw products in a platinum crucible at 1200°C. The molten glass was poured on a preheated brass plate in order to prepare I-CEL2 bars that were annealed at 375°C for 12 hours and then cut to produce slices. DSC analysis carried out on I-CEL2 powders showed the following characteristic temperatures Tg = 410°C, Tx = 590°C and melting above 670°C. On I-CEL2 slices *in vitro* tests were carried out in order to test their bioresorbability in distilled water, in tris-HCl and in SBF at 37°C for periods up to four months. During the soaking periods, the solution was refreshed twice a week and the pH variations were carefully monitored. At different soaking periods, I-CEL2 slices were removed from the medium, left dry overnight and then weighted in order to evaluate the glass resorption rate. The molten glass was also poured in water producing a frit that was subsequently ball milled in order to obtain powders that were sieved below 106μm. The sieved I-CEL2 powders were mixed with polyethylene particles within 100 and 300μm and then thermally treated to produce 3D-macroporous glass-ceramic scaffolds, as reported by the authors in a previous work for a silica-based glass [4]. At this purpose, cylindrical compacts of powders (greens) with a 30mm diameter were obtained applying 100MPa for 10 seconds. The greens were thermally treated at 550°C for 3 hours in order to remove the organic phase and to sinter the inorganic one. The obtained scaffolds were characterised through scanning electron microscopy (SEM), compositional analysis (EDS), X-ray diffraction (XRD) and *in vitro* tests in SBF. The scaffold porosity was assessed through image analysis on different scaffolds area and through weight measurements on 10 specimens.
In order to study the osteogenic potential of the proposed biomaterial, I-CEL2 powders were pressed and then sintered at the same conditions used for the scaffolds obtaining glass-ceramic disks that were cut in order to produce slices for cell characterisation. hMSCs were seeded on slices and cells morphology, viability, proliferation and function up to 2 weeks were assessed.

Results and Discussion

Fig. 1 reports an image of an I-CEL2 glassy slice before and after soaking for 4 months in distilled water at 37°C. As can be observed I-CEL2 noticeably resorpted with time through a superficial bioerosion process that did not affect the material integrity.

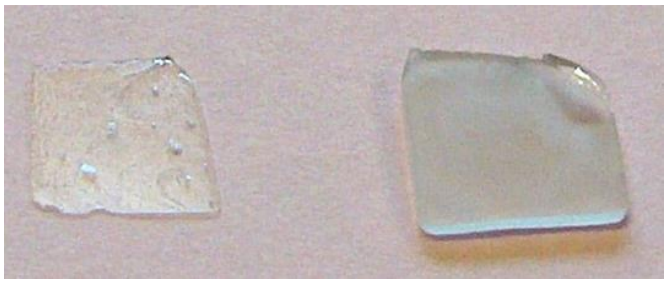

Fig. 1 I-CEL2 slice after 4 months of soaking in water (left side) and before soaking (right side)

The weight losses measured on I-CEL2 slices after 4 months of soaking in the different media are reported in Table 1.

Soaking medium	4 months (mg/cm^2)	4 months (%wt)
Tris-HCl	43.2	21.5
SBF	53.2	28.7
Water	136.0	75.9

Table 1 Weight loss of glass slices

A remarkable weight loss was observed in all media although lower weight losses were observed in Tris-HCl and SBF due to the formation on I-CEL2 slices of a white, thick layer as can be observed in fig. 2 for the samples soaked in Tris-HCl.
SEM observations were carried out on I-CEL2 slices after soaking in different media in order to investigate the bioresorption mechanism and eventual precipitation phenomena on their surfaces (data not reported). A bioerosion process was clearly observed on I-CEL2 slices soaked in water with the formation of cavity all along the glass surface; the EDS results demonstrated that the bioerosion was congruent as I-CEL2 slice did not modify its composition during time. As far as soaking in Tris-HCl and SBF are concerned, the observed white layer was studied through EDS analysis and showed to be rich in calcium and phosphorous.

Fig. 2 I-CEL2 slices after soaking in Tris-HCl for 4 months (left side) and before soaking (right side)

XRD analysis carried out on these I-CEL2 slices assessed the amorphous nature of the precipitated calcium phosphate layer; the formation of this layer is partially responsible for the different resorption rate observed in these two media. In fact the bioresorption is mainly due to cleavage of P–O–P bonds by water molecules that would diffuse more slowly through this precipitated calcium-phosphorous layer. 3D-macroporous scaffolds were successfully obtained with a good degree of reproducibility by mixing I-CEL2 powders with 50% vol. of polyethylene particles within 100 and 300μm; as an examples fig. 3 reports an image of the prepared scaffolds.

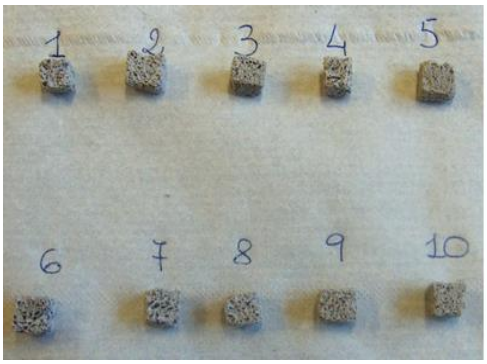

Fig. 3 Prepared 3D phosphate scaffolds

A total porosity of 90±1.5% was obtained demonstrating the high degree of reproducibility of the proposed method and the optimal characteristic of the obtained scaffolds. Fig. 4a reports a micrograph of the scaffold in which a structure with partially closed pores with thin walls and interconnected by open pores of a few tenths of microns can be observed; fig. 4b shows the results of an EDS analysis carried out on the whole area reported in fig. 4a.

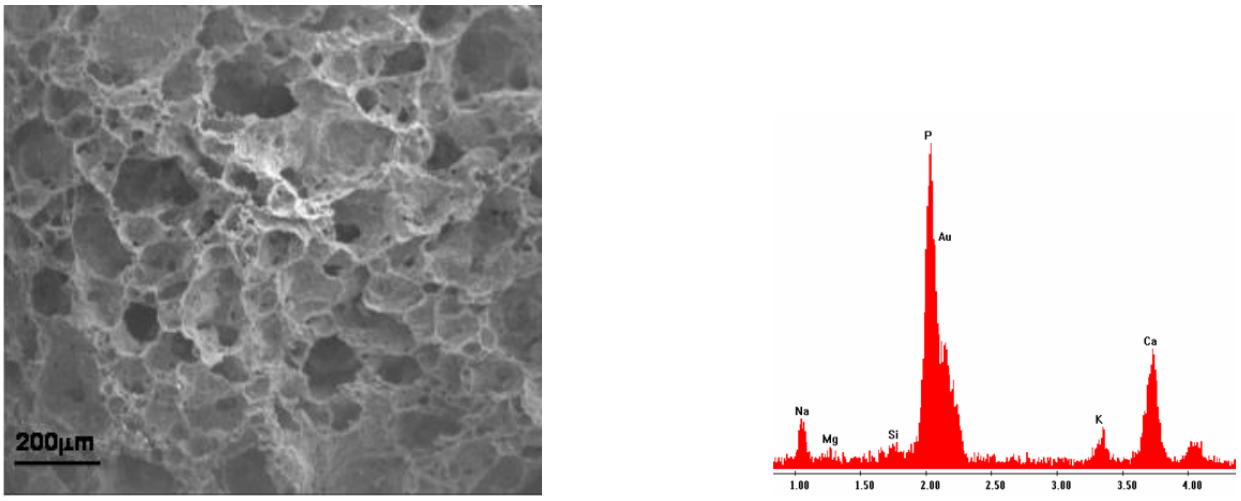

Fig. 4 I-CEL2 scaffold SEM micrograph (a) and EDS analysis of the scaffold surface (b)

The resorption of the scaffolds was tested by soaking 1x1x1cm^3 samples in SBF for periods up to 4 months. The total weight loss after four months was 75%wt. and any collapse of the scaffold structure was seen corroborating the hypothesis of a surface bioerosion process. During the bioresorption of the scaffold, the pH variations due to the ions release phenomena were monitored and the observed values varied within 7.30 and 7.40 which are perfectly compatible with cells viability.

For biological testing, I-CEL2 disks were pre-wetted for 2 h in fetal bovine serum-added medium and seeded with hMSCs. At early time-points hMSCs were observed to attach to the material surface, even if actin cytoskeleton is not yet organized (fig. 5)

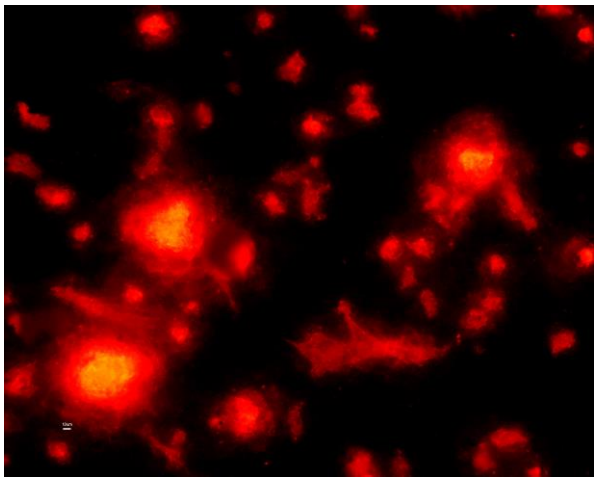

Fig. 5 TRITC-labelled phalloidin stained hMSCs on I-CEL2 slice at 24h (x10 magnification)

The pH of cells culture medium was not significantly altered in hMSCs-seeded samples.
The early adhesion rate was 30%, in comparison with tissue culture plastic-seeded hMSCs and the osteogenic phenotype was maintained, as shown by the intracellular alkaline phosphatase.

Conclusions

A new bioresorbable phosphate glass was successfully obtained and characterized as far as its resorption properties in different media are concerned.
I-CEL2 powders were used to produce 3D-macroporous scaffolds with a total porosity of 90% vol. and pores of a few hundreds of microns, partially open. The scaffolds are bioresorbed in SBF without collapse of the structure through a bioerosion mechanism. On these bases they should act *in vivo* as a temporary scaffold that will degrade during the bone re-growth without sudden loss of stability. The induced pH variations are negligible and preliminary *in vitro* tests with human cells encouraging.
On these bases the proposed materials can be suggested as valid scaffolds for guided bone regeneration.

References

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