Effect on rheological properties and 3D printability of biphasic calcium phosphate microporous particles in hydrocolloid-based hydrogels

Helena Herrada-Manchón [1,2,*], David Rodríguez-González [1,2], M. Alejandro Fernández [2], Nathan William Kucko [3], Florence Barrère-de Groot [3] and Enrique Aguilar [1]

[1]Departamento de Química Orgánica e Inorgánica. Universidad de Oviedo, Oviedo, Spain; [2]Idonial Technology Centre, Gijón, Spain; [3]Kuros Biosciences, Bilthoven, The Netherlands.

Introduction

Since the repair of large-area and complex-shape bone defects remains a significant challenge, 3D printing of on-demand bone graft biomaterials has been emerging as an alternative to autografts in the last years [1]. In that way, it has been widely reported that surface architecture, geometry and microporosity are essential factors for osteoconduction and, in some cases, osteoinduction [2,3]. Due to the latter, exploring the printability through semi-solid extrusion (SSE) of irregularly-shaped, microporous biphasic calcium phosphate (BCP) granules 150-500 µm in size and with a characteristic submicron surface structure is of particular interest. Due to the physical characteristics of the BCP particles (size, weight, and agglomeration trend), three well-known hydrocolloids (sodium alginate, xanthan gum and gelatin) have been combined to generate stable, homogenous, and printable solid dispersions which, to the best of our knowledge, have not been previously explored. For the obtained biomaterial inks, the relationship between the rheological behavior and the biomechanical properties of the hydrogels in terms of stiffness (elastic modulus) regarding the size/concentration of BCP granules and the printing parameters has been investigated [4].

Materials and Methods

The materials selected were sodium alginate (CAS no. 9005-38-3), gelatin (CAS no. 9000–70-8), xanthan gum (CAS no.11138–66-2), Dulbecco's Phosphate-Buffered Salt Solution 1X (DPBS) (Corning™ 21-031-CV).

Commercial biphasic calcium phosphate (BCP) bone graft (MagnetOs™ Granules) was kindly provided by Kuros Biosciences BV as 150-500 µm size particles (BCP1) and 150-250 µm size particles (BCP2). Four biomaterial inks were prepared by mixing the same hydrogel with different ratios and particle sizes of BCP (Table 1).

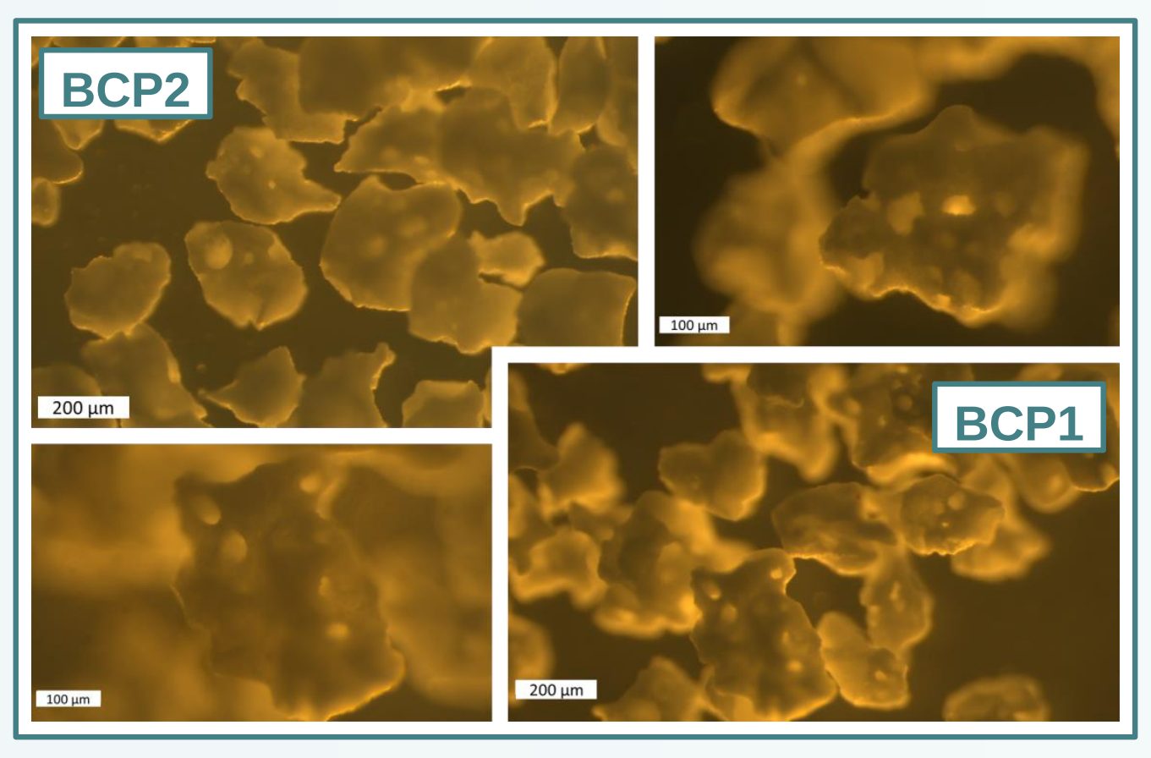

Fig.1. BCP particles

	wt%			
	30BCP1	15BCP1	30BCP2	15BCP2
Xanthan gum	3	3	3	3
Alginate	2	2	2	2
Gelatin	2	2	2	2
BCP1	30	15	-	-
BCP2	-	-	30	15
DI water	63	78	63	78

Table 1. Biomaterial inks formulations

Rheological characterization of ink samples (flow behavior, thixotropy, and stiffness) was carried out with a DHR-2 rheometer equipped with a parallel plate (25 mm) and a controlled heating oven for stable temperature control. A stepper motor-driven syringe-based extrusion 3D printer (bIDO-I, Idonial Technology Center) was used to print the figures (Fig.2.). The swelling ratio (SR%) of BCP hydrogels was calculated in freshly crosslinked cubes and dehydrated cubes to evaluate the water absorption in post-processed structures (Eq.1).

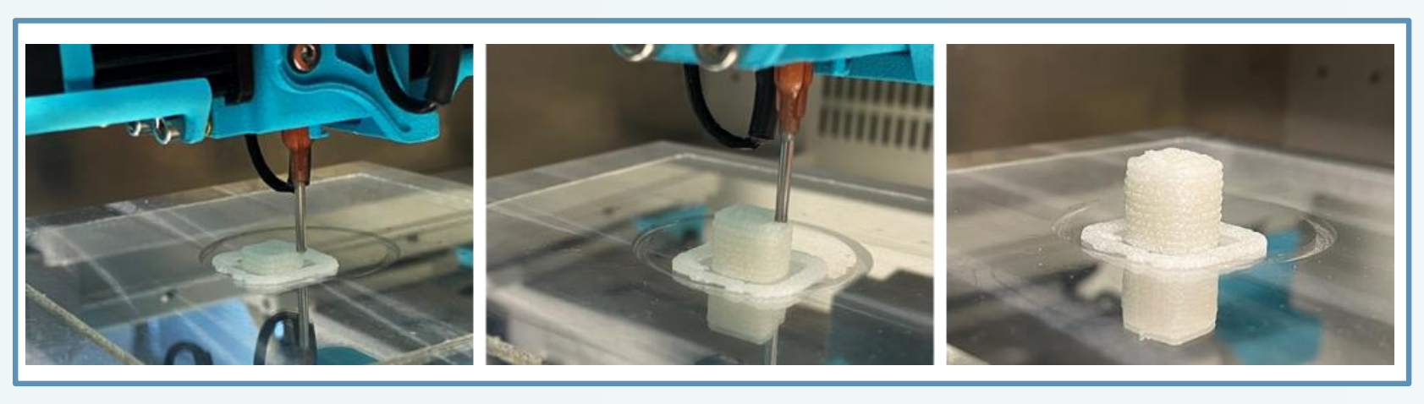

Fig 2. 3D printing process

$$SR\% = \frac{(W_f - W_i)}{W_i}\ x\ 100$$

Eq.1. SR% calculation

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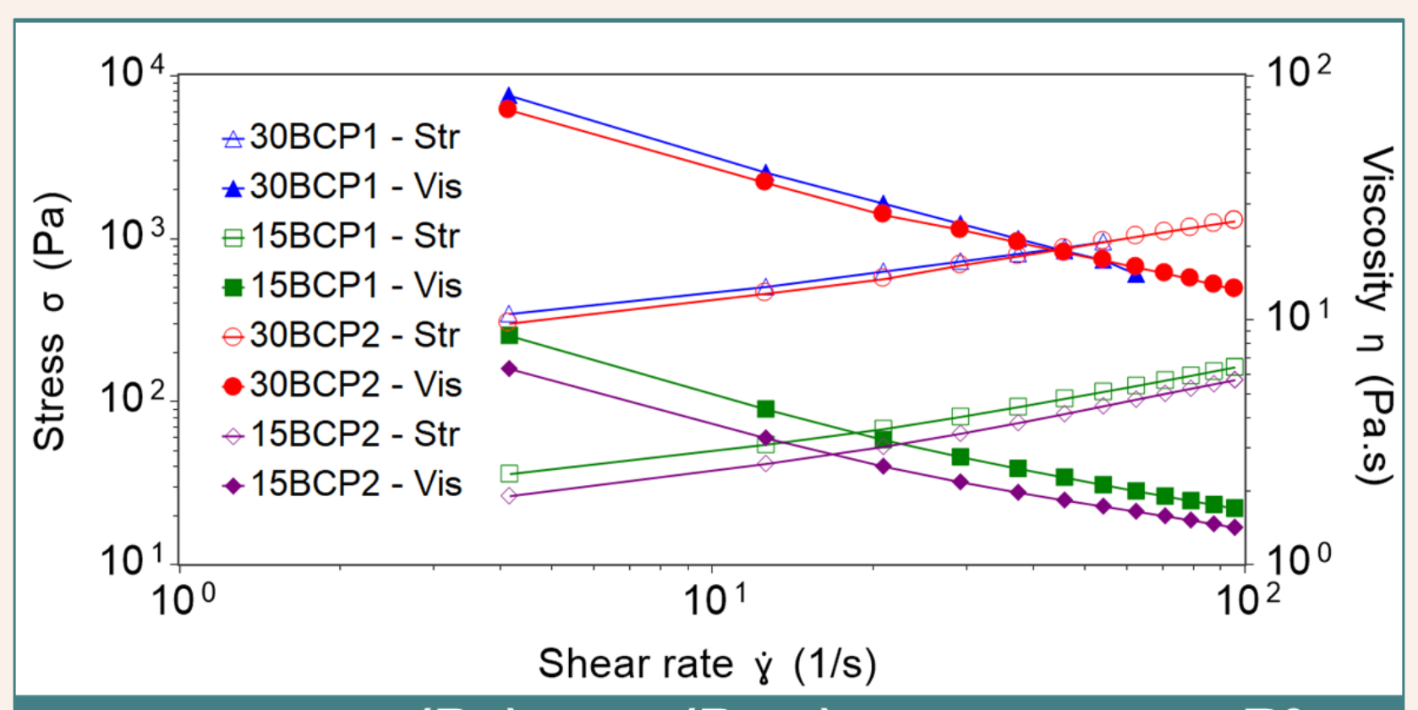
Results and Discussion

All the mixtures showed a decrease of viscosity when increasing shear rates, revealing clear shear-thinning properties of the BCP-hydrogels. Flow curves were similar for inks with equal particle concentration although particle size was different. However, 30BCP1 became shear-thickening at high shear rates and suffered jamming. The Herschel-Bulkley (H-B) mathematical model fit the experimental results with R² values higher than 0.999 for all the inks. For the same particle concentration, the larger the size of the granules, the higher the yield stress and *K* value, denoting an increase in apparent viscosity. Similarly, the mixtures with a higher quantity of particles had a lower *n* value, and therefore, a greater pseudoplastic behavior (Fig.3.).



	τ_0 (Pa)	K (Pa.s)	n	R²
30BCP1	142.08	93.25	0.54	0.9999
15BCP1	21.92	4.98	0.73	1.0000
30BCP2	125.46	70.38	0.62	0.9993
15BCP2	15.38	3.66	0.77	0.9999

Fig.3. Flow curves and H-B parameters

Thixotropy was quantitatively measured through a Stepped Dynamic Method (SDM). The viscosity recovery values ranged between 72-89% for the different biomaterial inks, values that allowed for a controlled deposition of the ink and a faithful execution of the models designed during 3D printing (Fig.4.). As the flow curves predicted, printing with 30BCP1 was not suitable as caused nozzle clogging with the shear (extrusion) regardless of the temperature tested.

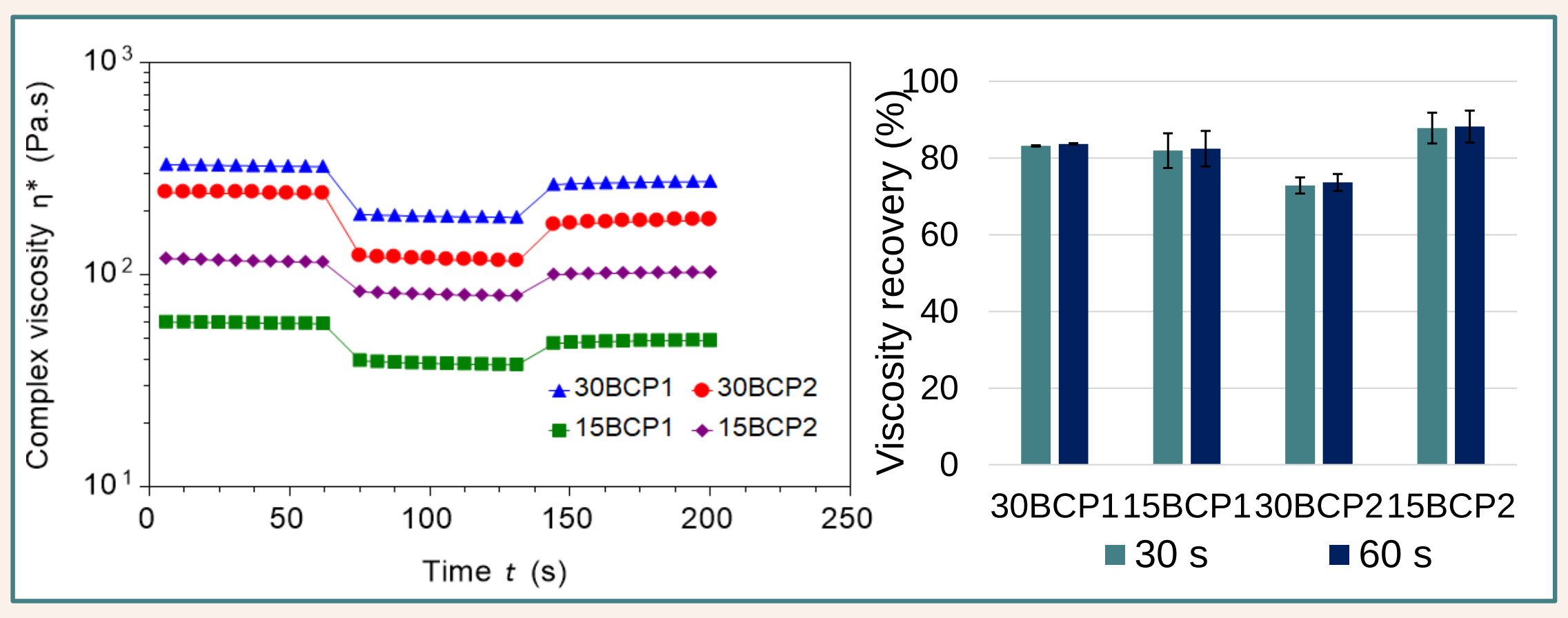


Fig.4. SDM and viscosity recovery (%) at room temperature

By combining two infill patterns (rectilinear (R) and honeycomb (HC)) with two infill densities (60% and 75%), four different structures were configured and executed precisely with the three printable inks (Fig.5.). Microscopic observation of the figures showed that the BCP particles were uniformly distributed in the extruded filaments. Once deposited, the printed lines remained fairly defined and could be clearly distinguished, especially in the case of 30BCP2.

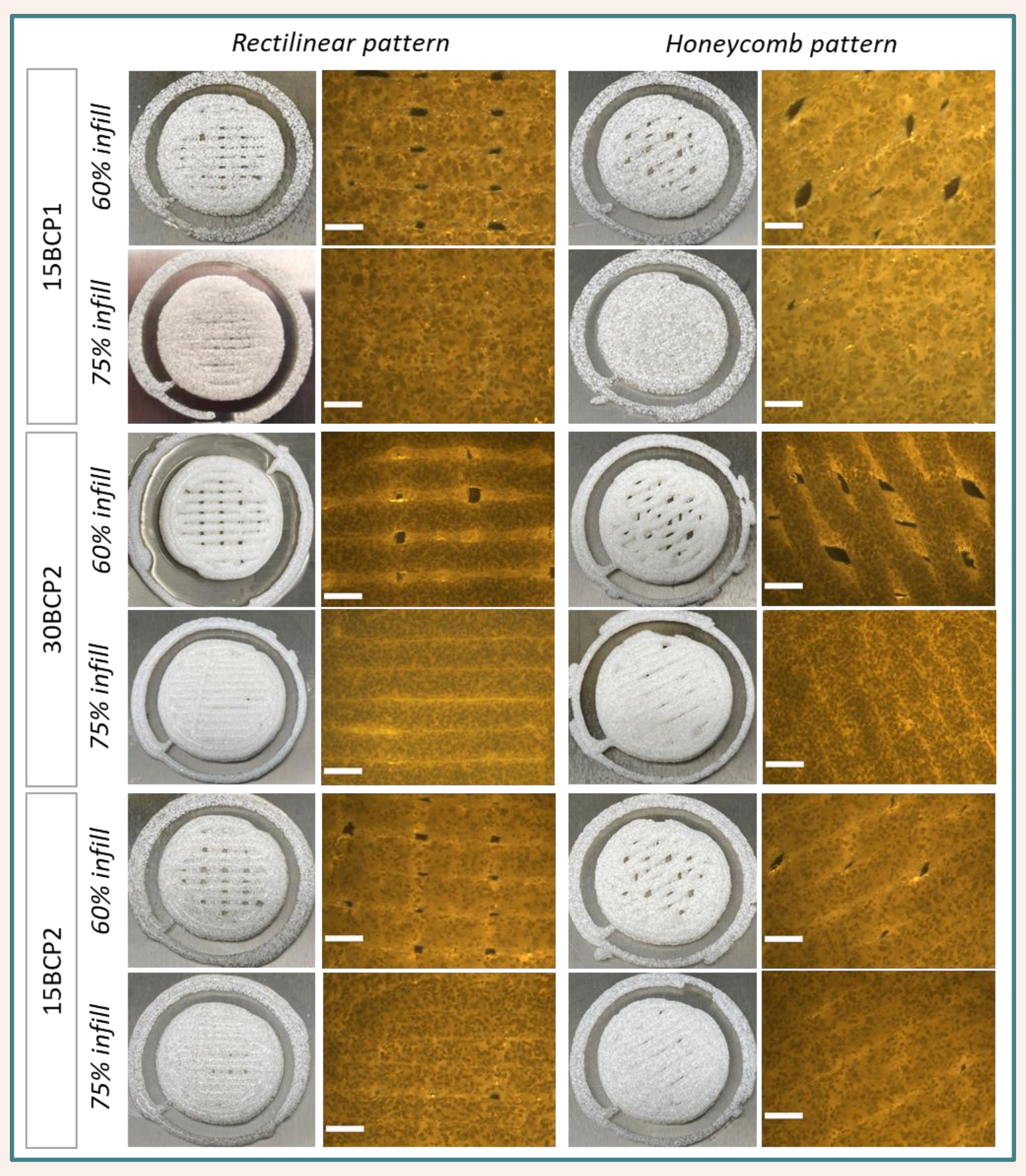


Fig.5. 3D printed disks. Scale bars: 2 mm

Regarding the stiffness of the matrix, by comparing the values of the elastic or storage modulus (*G'*) at 1% strain, it was observed that the highest results detected corresponded in all cases to the 30BCP2 ink, regardless of the pattern or infill percentage set. However, for the same pattern, the less filled figures had higher stiffness. This effect was because more porous structures have a greater contact surface when crosslinking with the alginate and therefore, more rigidity (Fig.6.). Thus, it was evidenced that not only the ink composition but also the printing parameters greatly influence the biomechanical properties of the printed structures. Consequently, they must be taken into account and evaluated because, as other authors have already demonstrated, the function of cells residing in bone tissue could be affected by material stiffness.

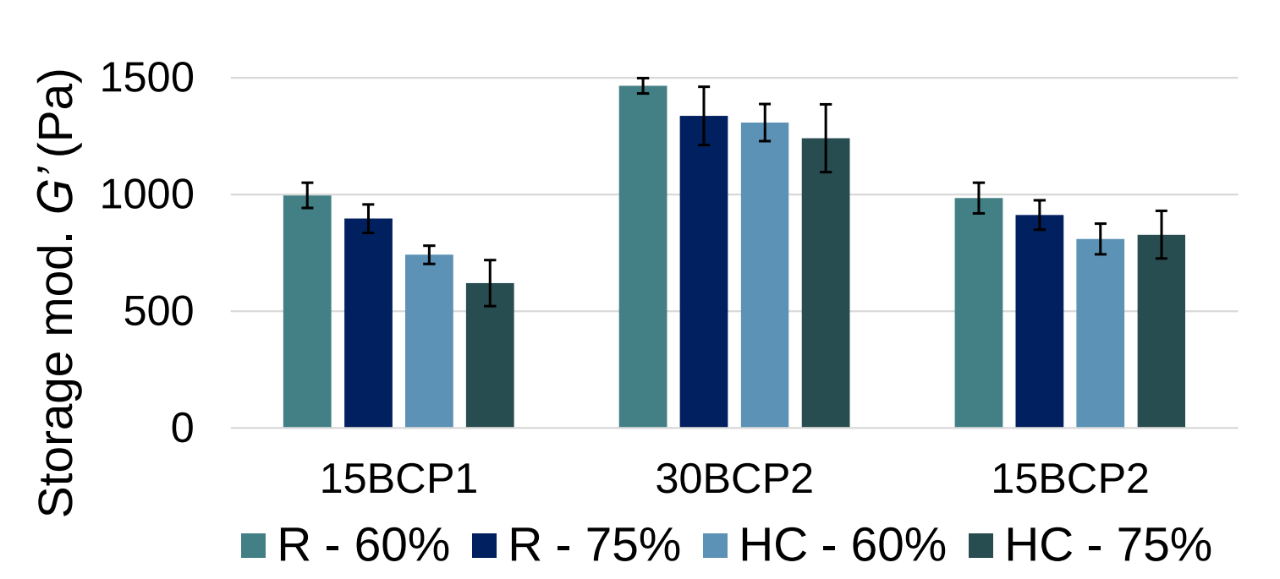


Fig.6. *G'* values detected at 1% strain for R or HC patterns at 60% or 75% infill

Finally, 10 mm-edge cubes were printed with 1 mm of layer height, a rectilinear pattern and 60% infill. The swelling ratio of the fresh-printed and dehydrated structures was analyzed in calcium-free DPBS. For fresh samples swelling profiles differed with respect to granule size, as smaller particles (BCP2) were faster released from the hydrogel matrix. Regarding the dehydrated samples, the cubes printed with 30 wt% particles underwent a faster disintegration during the rehydration process. In that case, the production of stronger crosslinked matrices should be devised (Fig.7.).

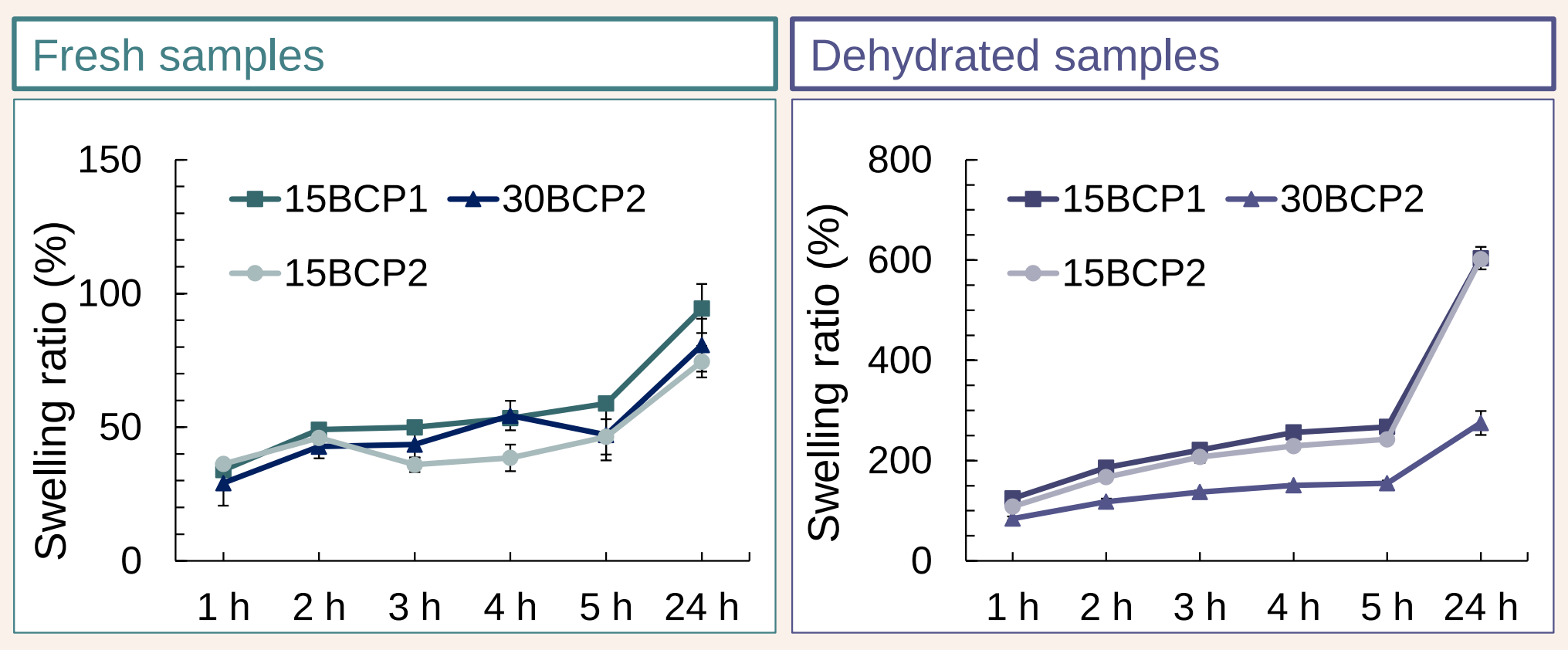


Fig.7. SR% for fresh and dehydrated samples

Conclusions

- A new ceramic biomaterial ink has been generated by combining BCP particles and an alginate/gelatin/xanthan gum-based hydrogel.
- Concentration and particle size have a significant influence on rheological and mechanical properties.
- Different levels of stiffness and rigidity are achieved based on the configuration of the printing process parameters.
- The swelling behavior is influenced by the matrix composition and the post-processing applied to the printed structures such as drying procedures.

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linkedin.com/in/hherrada @HelenaHerrada helena.herrada@idonial.com