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New Bone Tissue Engineering Approach With Osteoinductive Biomimetic PLA/N-Methyl-2-Pyrrolidone Scaffold Enriched With Lyophilized Gelatin Granules And Hydroxyapatite Microparticles. In Vitro And In Vivo Study. Preliminary Results.

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BACKGROUND AND AIM

A wide range of pathological phenomena often generate Critical Size Defects (CSDs) in the jaws which requires reconstructive surgery procedures for morphological and functional recovery. Over the last 50 years a considerable variety of products and technique have been proposed, but today the scientific community agrees that the "gold standard" to treat these volume losses are the autologous bone grafts. However, there are several disadvantages using autologous bone as a need for a second surgical site and greater patient morbidity and today, the alveolar bone defects reconstruction remains an open clinical challenge. To overcome of CSDs problems, the bone tissue engineering (BTE) strategy is to create a very complex structure, called scaffold, which might mimic the conditions of the bone microenvironment and fill the limits of current reconstructive surgical therapies.
The aim of this research project was to create innovative biomimetic composite bone scaffold consisting of a mixture of PLA, HA and gelatin dissolved in an N-methyl-pyrrolidone solvent and evaluate its mechanical characteristics, in vitro and in vivo biocompatibility and osteoinductive ability.

MATERIALS AND METHODS

Different formulations were initially evaluated based on the concentration of the constituents in order to identify the most performing scaffold under mechanical profile. To evaluate the mechanical properties, cylindrical acellular scaffolds were created (6 x 12 mm) and uniaxial compression (N), compressive stress (MPa) and Young module (MPa) tests were determined at 10%, 25% and 50% of deformation. Subsequent analyzes were performed on the most mechanically performing scaffold only. Rheological analysis, washout resistance, optical and scanning electron microscopy were performed. *In vitro* phase was carried out using adipose stem cells (ASCs), ascertained by flow cytometry analysis. ASCs were obtained from human retropatellar Hoffa's fat-pad samples. AD-MSCs were grown with or without scaffold in normal growth medium or osteogenic medium respectively and the RNA extracted at 5th and 45th day of culture for quantitative Real-time PCR evaluation (RUNX2 and OSX). To test *in vivo* scaffold biocompatibility, the model of the chicken CAM was used. The experiments started on the 7th day of development (time point: T1) and all embryos, six replicates for each sample, were monitored for up to 72 hours (time point: T3). In the in vivo phase CAM reaction was evaluated with vascular semiquantitative index and survival rate. After the sacrifice, optical microscopy examinations were conducted. Data analysis was conducted using statistical software (STATA) to evaluate any statistically significant difference between the test and control groups at different time points. The cutoff value for determining statistical significance corresponding with a p-value of 0.05 (5%) or less..

Figure 1: Scaffolds synthesis

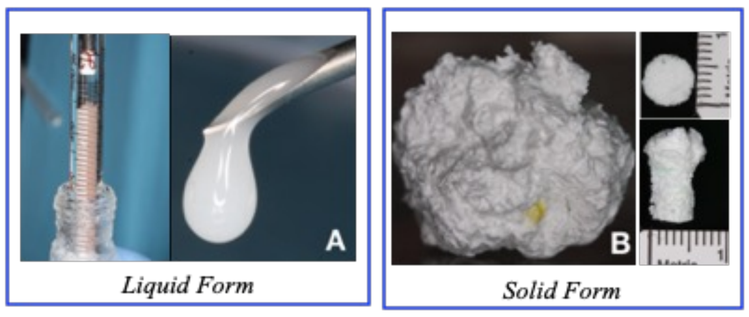
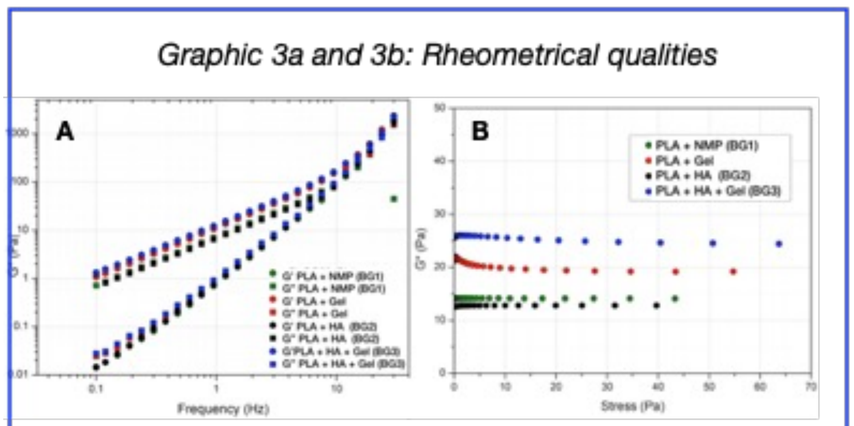


Table 1: Scaffolds composition

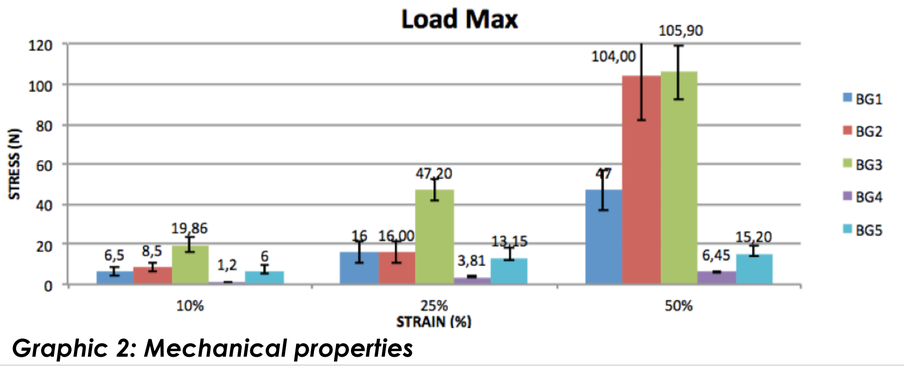
Scaffold Code	PLA	NMP	Ca^{++} Phosphates	Natural Polimer
BG1	0.88 g (46,07%)	1 mL (53,93%)	-	-
BG2	0.88 g (42,10%)	1 mL (49,28%)	HA 0.18 g (8,61%)	-
BG3	0.88 g (38,77%)	1 mL (45,37%)	HA 0.18 g (7,93%)	Gelatin 0.18 g (7,93%)
BG4	0.88 g (38,77%)	1 mL (45,37%)	HA 0.18 g (7,93%)	Algintate 0.18 g (7,93%)
BG5	0.88 g (42,10%)	1 mL (49,28%)	β-TCP 0.18 g (8,61%)	-

Graphic 3a and 3b: Rheometrical qualities

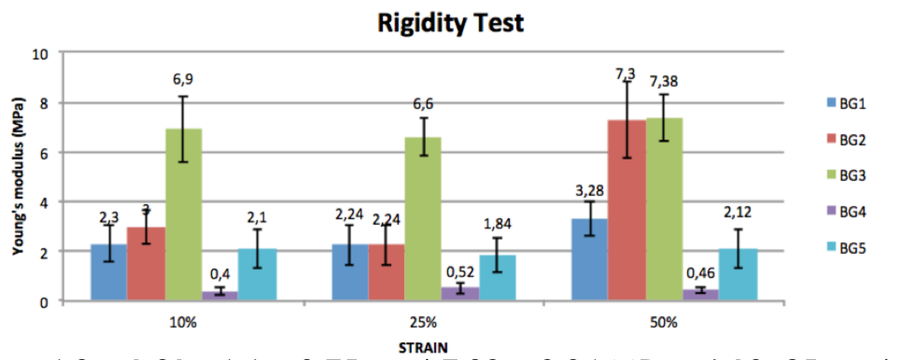


Sample	Viscosity
BG1	13 ± 2 Pa*s
PLA + Gel	20 ± 3 Pa*s
BG2	12 ± 3 Pa*s
BG3	26 ± 2 Pa*s

Graphic 1: Mechanical properties



Graphic 2: Mechanical properties



RESULTS AND CONCLUSIONS

Results: Scaffold formulation called BG3 showed the best mechanical performance with a Young's Modulus 6.9 ± 1.31, 6.6 ± 0.75 and 7.38 ± 0.94 MPa at 10, 25 and 50% of deformation, with an average porosity value of 57%. In the liquid form BG3 showed a viscosity of 26 ± 2 Pa * s at 37 ° C with rheometrical qualities of a weak gel. Both the liquid and solid formulations are highly biocompatible in both in vivo and in vitro evaluation at T1 and T3. RT-PCR analysis showed better Runx2 expression at 5 days than the control group. The expression of OSX was not statistically different between BG3 in Normal growth medium than BG3 in osteogenic medium at 45 days of culture, but the two values were significantly higher than the baseline. In the present study, a new type of biomimetic composite scaffold has been synthesized, characterized and tested in vivo and in vitro. Its application in bone regeneration and bone tissue engineering procedures could be useful thanks to the possibility of use both in liquid and solid form. The qualities in terms of biocompatibility both in vivo and in vitro were excellent despite the concentrations of NMP used. The mechanical characteristics did not show ideal values compared to what was required of an ideal bone scaffold, but anyway sufficient in the management of problems commonly found in GBR procedures. The use of BG3 is not desirable for blocking free bone fragments subject to load but only as bone filler in CSDs in non-mobile bone segments. It is conceivable

Figure 2: In Vitro study time line

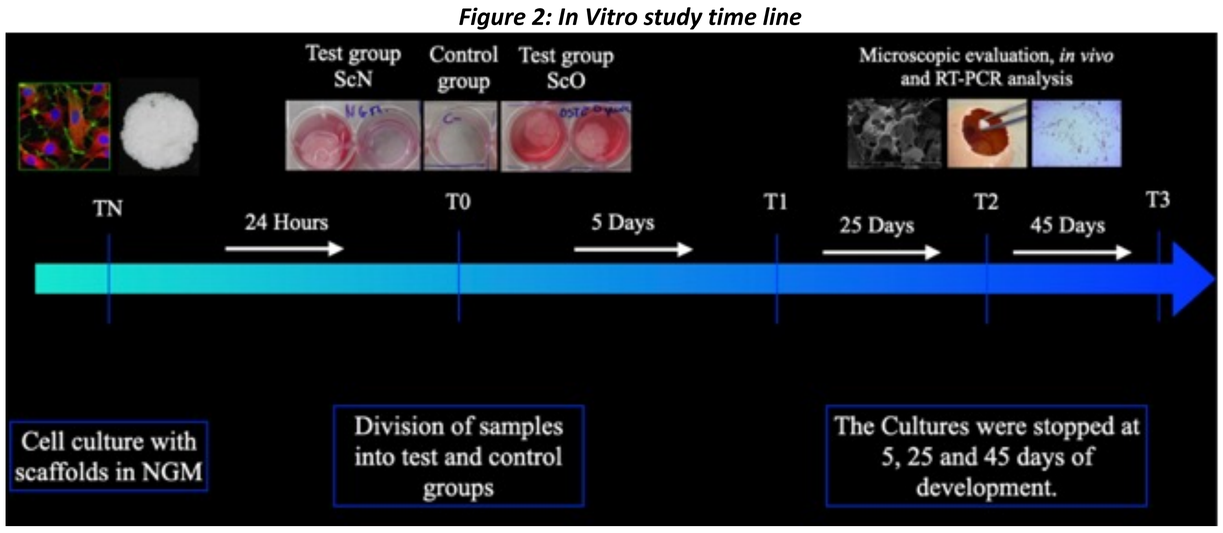
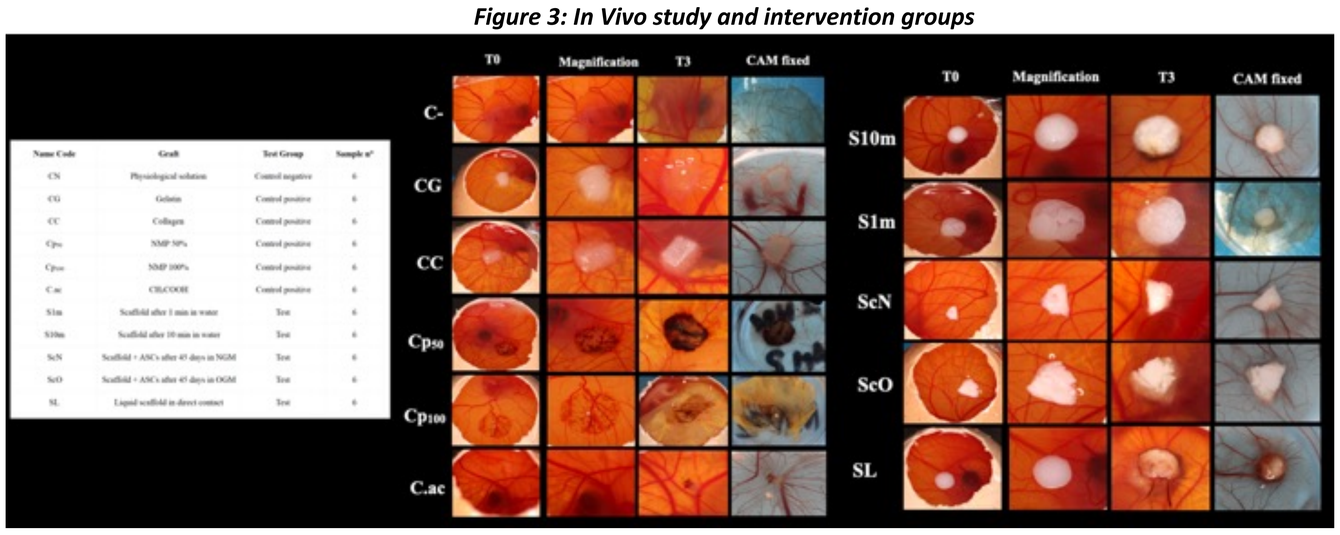


Figure 3: In Vivo study and intervention groups



Graphic 4: Gene expression

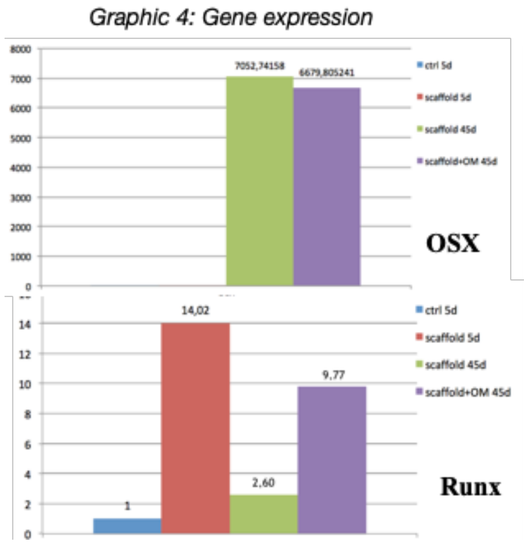


Figure 4: Vascular index evaluation

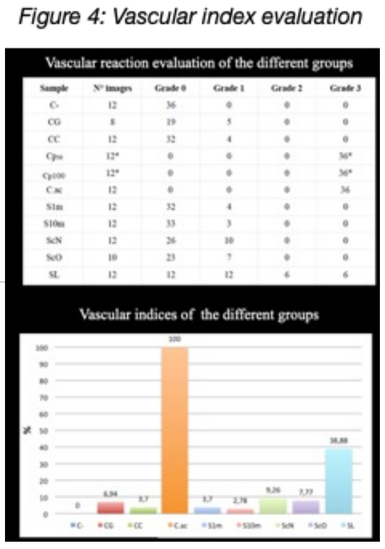


Figure 5: Histological section of the ScO (above) and SL (below) scaffold. E-E staining

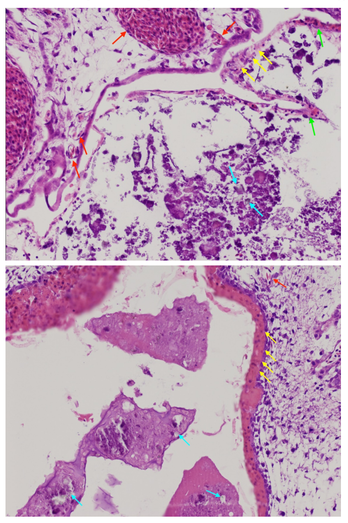


Figure 6: Survival analysis from T1 to T3