European Review for Medical and Pharmacological Sciences 2023; 27 (3 Suppl): 153-161

Wettability of Mg-HA/Chitosan-based membrane surfaces: blood *vs.* autologous platelet liquid (APL)

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Abstract. – OBJECTIVE: The physical and physical chemistry is able to influence the interaction of the scaffolds and bone substitutes with the body fluid and blood. The aim of the present investigation was to evaluate the wettability properties of an Mg-HA Chitosan-based Gel with blood vs. autologous platelet gel.

MATERIALS AND METHODS: A total of 6 study groups were evaluated according to the Mg-HA Chitosan-based Gel thickness (1, 2 and 3 mm) and the fluids (blood *vs.* autologous platelet gel). The biomaterial wettability was conducted through the sessile drop technique.

RESULTS: The study findings showed a significant difference in contact angles between the APL and blood groups ($p<0.05$). The MG-Ha Chitosan-based membrane thicknesses seem to produce no significant effects on contact angles measurement for all groups ($p>0.05$).

CONCLUSIONS: In the present investigation, a similar MG/Ha gel membranes wettability was reported between APL and blood groups. In addition, a high hydrophilicity of MG/Ha gel membranes was reported with a potential advantage in terms of a more effective osteogenic capability in the clinical practice.

Key Words:
Blood platelets, Platelet derived growth factor, Dental implant, Osseointegrated dental implantation.

Introduction

The first line of defense against any bacterial-virus invasion in oral cavity is mucous membranes[1]. Often, there are several wound in this site but, in physiological condition, they should completely recover in few days[2]. If the healing requires a time greater than 12 weeks they are defined as chronic wound sites. The increased risk of bacterial invasion and tissues infection could inficiate the wound healing[2,3]. Up to the present day, the knowledge of intraoral healing is limited, which reduces the clinical translation of treatment alternatives. Moreover, we have to take into account that in case of impaired wound healing, the oral cavity can be susceptible to challenges that arise from trauma-related injury, prolonged inflammation, and postoperative complications[2,4,5]. In this context, the regenerative-based approaches hold out promise to enhance oral wound healing and require targeted treatment options to improve tissue re-epithelialization and extracellular matrix (ECM) remodeling[2,3]. Main of these technologies are based on the use of biomaterials that are able to release biological factors and to drive the cells for tissue regeneration[6]. The material choice is extremely important in establishing good tissue synthesis and postoperative success. Scaffolds productions aim to accelerate the healing process, prevent bleeding, and contribute to forming and maintaining the blood clot, be biocompatible, not cytotoxic, and avoid infection and inflammation at the site[7]. Moreover, they require increased adhesive properties to be de-

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graded solving as space maintainers at the level of the surgical site[7]. Using highly innovative biotechnological approaches, the present work aims to open up new and promising paths for the treatment of difficult-to-heal and severely disabling skin and mucosal lesions[8]. These serious clinical problems may result from infections, diabetes, immune diseases, trauma, amputations, or post-operative complications. In the first place, they are one of the most serious and disabling consequences of various neglected diseases (e.g., leprosy, bullous diseases, zoonoses), which have mostly disappeared in the West but are still widespread in tropical and developing countries, where healthcare systems are highly inadequate in the prevention and treatment of such diseases[8-10]. In this work, the authors tested new-generation skin grafts made with biomimetic implants, capable of reproducing the various layers of the skin while completely integrating until they reabsorb and disappear entirely. The new grafts are mineralized with bioactive and antibacterial nanocrystals so that they exchange chemical signals with the damaged skin or mucous membranes that promote physiological cell metabolism, neo-vascularization, and tissue regeneration[6]. In addition, the new grafts are designed to absorb and release autologous haemocomponents into the injured part; these substances, constantly self-produced by our organism, are rich in growth factors and substances capable of promoting, supporting, and enhancing natural self-healing mechanisms[11,12]. In this preliminary works, in order to evaluate the ability of the scaffold to be embedded by growth factors, signals, and exosomes present in haemocomponents, we tested *in vitro* their wettability.

Materials and Methods

MgHa-Chitosan Hybrid Gel Patches

The materials are presented as beige-colored porous patches. They consist of a biomaterial gelatin/chitosan-based hybrid biomaterial, in a ratio of 70/30 wt%, added with 30 wt% of mineral component with antimicrobial function represented by hydroxyapatite replaced with ion-mineralized magnesium on gelatin (MgHA- Chitosan Gel). The samples underwent a process of freeze-drying and cross-linking in a vacuum at 120°C for 24h in order to ensure good porosity and stability in a physiological environment as well as optimal ability to swell and absorb medium or haemocomponents. Samples size is about 2-3 mm and thinner by about 1 mm, in order to be able to test both of them.

Preparation APL

Blood sampling was carried out from six patients with average age 53 years, range 45-57, non-smoker, with no history of hemorrhagic diathesis, coagulopathies, medication intake, serious systemic diseases and with physiological blood parameters. The number of platelets of the donor was 264,000 platelets/μL, (range from 150,000 to 351,000) taken from previous blood tests. In oral implantology, blood is usually used for mixed bone graft for achieving sticky graft[13], or to produce autologous platelet gel/liquid and mixed with biomaterials. For various cases of oral surgery, blood was used for our *in vitro* study or to produce autologous platelet liquid. The blood samples were obtained by venipuncture from the median cubital vein using a 21-G butterfly needle and a pre-assembled support with Luer Lock attachment. Blood was collected in 9 ml blue vials with anticoagulant test tubes, containing a solution of 3.8% sodium citrate and one red vial. Blood fractionation was performed with a GFONE centrifuge (Ubgen Srl, Padua, Italy), with a 1.751 speed (RPM) and relative centrifugal force (RCF) of 246 for 7 minutes at room temperature. After centrifugation, the blood samples were divided into three layers: red blood cells on the bottom, plasma in the upper part of the tube and the buffy coat interposed (Figure 1 A-D). A 2.5 ml syringe with a long needle (70 mm) was used to aspirate the layer rich in platelets and fibrinogen, above the buffy coat, obtaining approximately 0.5 ml of APL per tube. The APL was activated with 500 μL of autologous thrombin aspirated from the top layer of red vial and 50 μL of the calcium chloride (CaCl2). The wettability of the implant surfaces was assessed with the sessile drop technique, using two different biological fluids: whole human blood, without the addition of anticoagulant on 5 samples for each type of surface and APL on the same number. 5 microliter drop was released perpendicularly with a few mm distance onto the titanium sample surface. High-magnification images were captured within a few seconds of placement, when the drop was in equilibrium, with a Nikon high resolution camera (Nikon D90). The measurements were made in the same environmental conditions, at

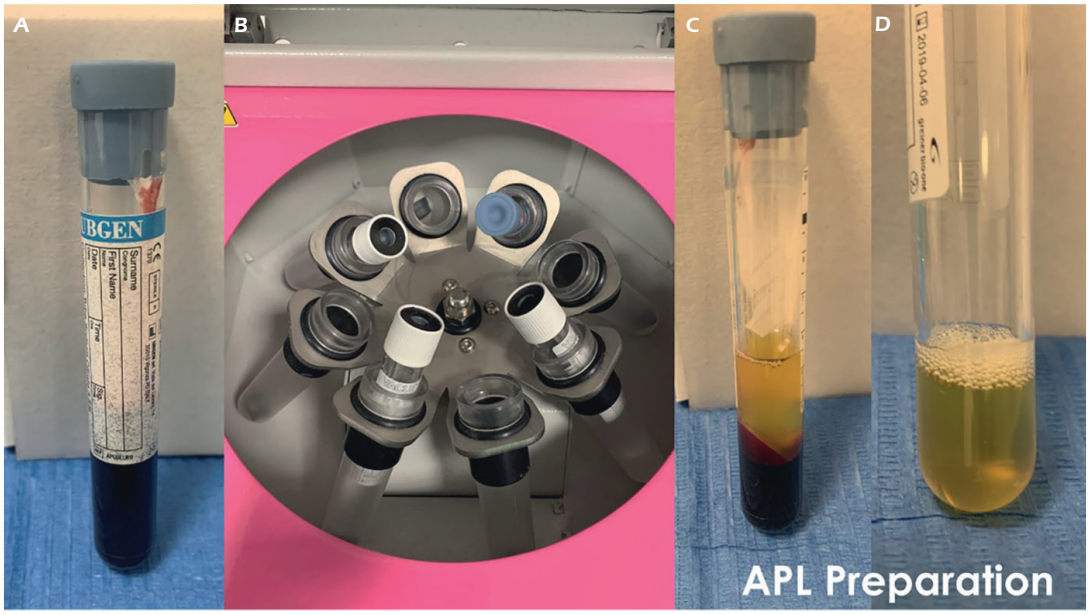

Figure 1. Detail of the Autologous Platelet Gel (AGP) preparation. **A**, Blood collected; (**B**), Vaquette in position in centrifuge; (**C**), Emocomponent fractionized; (**D**), Autologous platelet liquid separation.

room temperature (T = 23 ± 2˚C) (Figures 2 A-D and Figure 3 A-D). A graphic image processing software (Image J 1.47 v Wayne Rasband, National Institute of Mental Health, Bethesda, MD, USA) was used to calculate the CA angle from the average of the right and left sides of

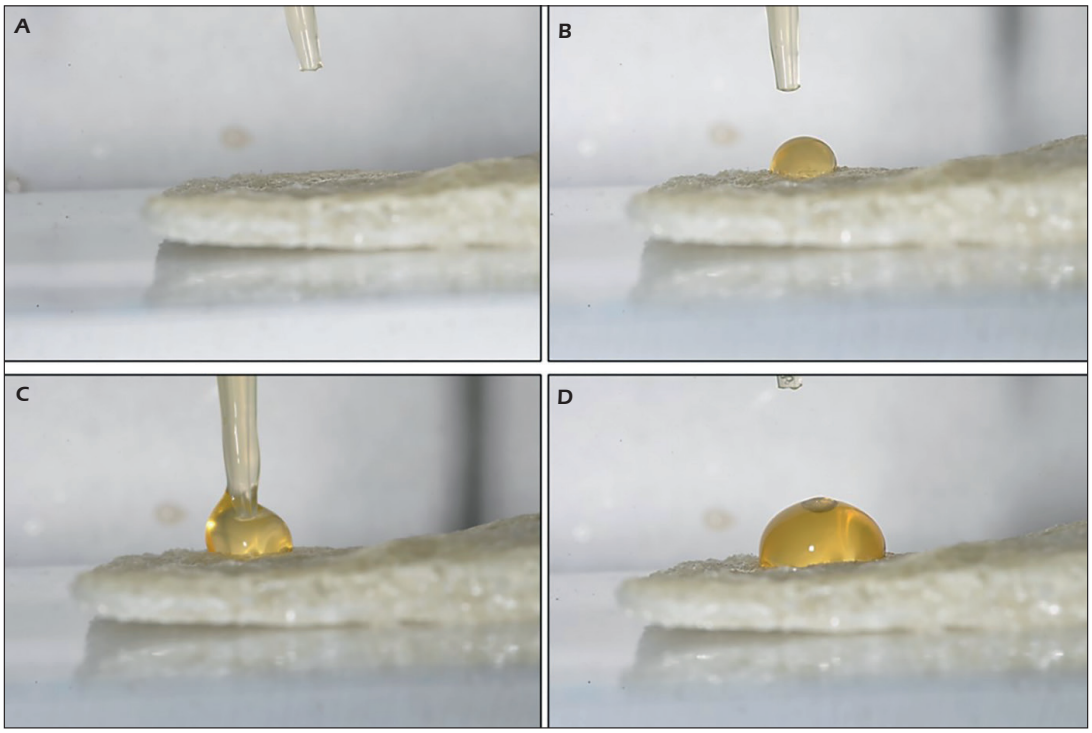

Figure 2. Frames details of the sessile drop technique experiment: MgHA-Chitosan-based gel and APL samples. **A**, Frame 1; (**B**), Frame 2; (**C**), Frame 3; (**D**), Frame 4.

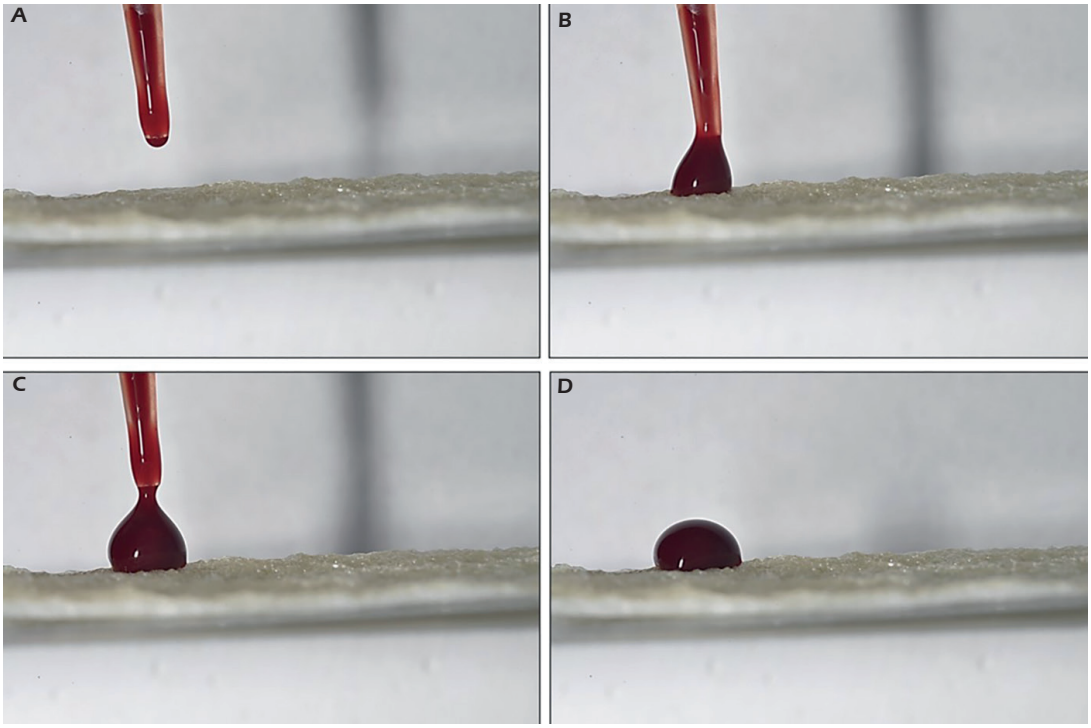

Figure 3. Frames details of the sessile drop technique experiment: MgHA-Chitosan-based gel and Blood samples. **A**, Frame 1; (**B**), Frame 2; (**C**), Frame 3; (**D**), Frame 4.

the drop, thus obtaining 1 value per sample. For the qualitative evaluation of the clot and fibrin structure, drops of both biological fluids were homogeneously distributed on the surface of the samples. After coagulation, the samples were rinsed with 5% buffered glucose solution and were fixed overnight in a buffered solution at pH 7.2 of 10% formalin. The samples were washed again with buffered glucose solution, dehydrated in an ascending alcohol series (50%, 80%, and 100%), dried and metallized in Emitech K 550 (Emitech Ltd) and observed with SEM. For each sample microphotographs were taken at different magnifications.

Study Design

The MgHA gel membranes were classified in a total of 6 different groups:

1) Group I APL – 1 mm Membrane Gel MgHA.
2) Group II APL – 2 mm Membrane Gel MgHA.
3) Group III APL – 3mm Membrane Gel MgHA.
4) Group IV Blood – 1 mm Membrane Gel MgHA.
5) Group V Blood – 2 mm Membrane Gel MgHA.
6) Group VI Blood – 3 mm Membrane Gel MgHA.

A total of 10 measurements for each study groups were performed.

Wettability Measurements

The membranes wettability was performed by the sessile drop technique, using the fresh blood and APL solution. A drop of 100 microliters was obtained by a calibrated mechanical pipette and dropped to the surface of the sample. High-magnification images were captured when the drop was at equilibrium at three different timepoints: 0 seconds (T_0), 30 seconds (T_1) and 60 seconds (T_2) using a high-resolution camera with macro-lens system (D90, Nikon, Tokyo, Japan). The conditions were carried out in the same stabilized environmental conditions (T = 23 ± 2°C). The high-resolution images were processed by a dedicated software package (Image J 1.47 v Wayne Rasband, National Institute of Health, Bethesda, MD, USA) in order to calculate the CA angle, as the mean of the right side and that of the drop. The mean value per sample was considered for further statistical evaluation.

Statistical Analysis

The statistical analysis was carried out using a statistical analysis software (Graphpad 6 Prism, San Diego, CA, USA). The descriptive statistics was performed considering the means and stan-

Table I. Descriptive statistics of the CA of AP /Membrane Gel MgHA groups and Blood/Membrane Gel MgHA groups [$p < 0.05$; Welch and Brown-Forsythe followed by the Games-Howell's post hoc test].

APL groups	Group I APL/1 mm membrane gel MgHA		Group II APL/2 mm membrane gel MgHA		Group III APL 1 mm membrane gel MgHA	
Timepoints	Mean	SD	Mean	SD	Mean	SD
0s	91.00	4.24	105.50	9.19	94.50	0.71
30s	90.00	4.12	104.00	11.31	93.50	0.71
60s	83.50	4.95	102.00	9.90	91.00	1.41
Blood groups	**Group IV blood/1 mm membrane gel MgHA**		**Group V blood/2 mm membrane gel MgHA**		**Group VI blood/3 mm membrane gel MgHA**	
Timepoints	Mean	SD	Mean	SD	Mean	SD
0s	98.04	15.56	108.52	2.82	105.36	9.19
30s	96.53	16.26	107.55	2.90	105.23	4.24
60s	93.57	13.43	107.27	2.12	104.35	4.42

dard deviations of all study groups. The Welch and Brown-Forsythe followed by the Games-Howell's post hoc test was performed. The level of significance was considered for $p<0.05$.

Results

The contact angles (CA) of the MgHA gel membranes with APL (groups I-II-III) or fresh blood of (groups IV-V-VI) was presented in Table I. The APL/Membrane Gel MgHA membranes showed lower contact angles if compared to the Blood /Membrane Gel MgHA ($p<0.05$). Considering the membranes thickness, no significant difference was present comparing the APL/Membrane Gel MgHA groups at all timepoints ($p<0.05$) (Table I, Figure 4). Moreover, no significant difference was detected comparing the Blood /Membrane Gel MgHA groups at all timepoints ($p<0.05$).

APL/Membrane CA Timepoint Variations

The APL/1 mm membrane showed a mean percentage variation of the 1.09% after 30s ($p>0.05$) and 8.24% after 1 minute ($p<0.05$). Similar results were reported by APL/2 mm membrane with an average variation of 1.42% ($p<0.05$) after

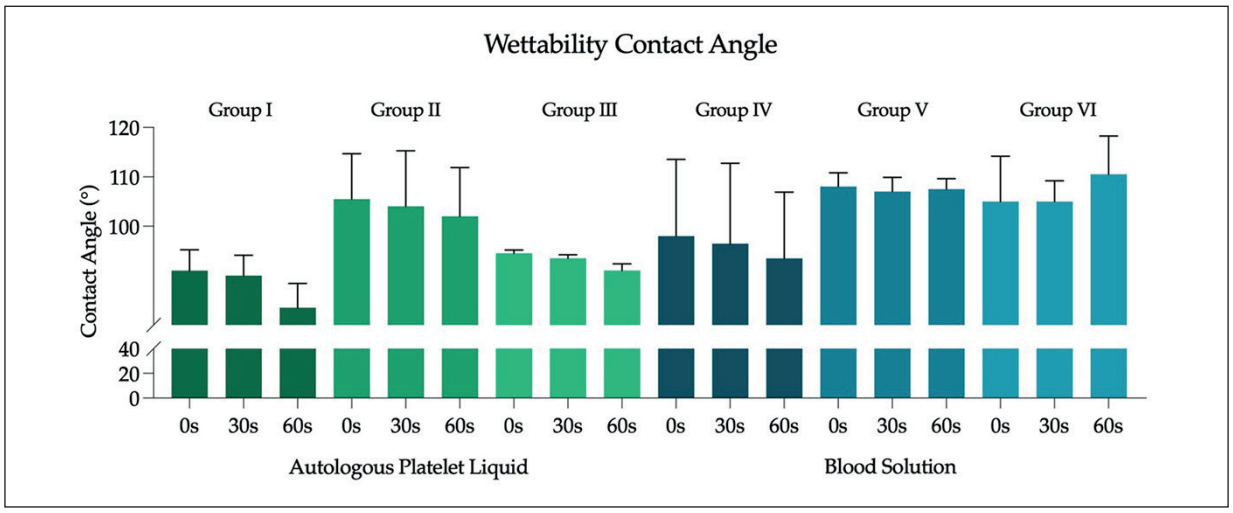

Figure 4. Wettability contact angle (CA) of APL/membrane Gel and blood/membrane Gel groups. For all membranes ticknesses, the APL/Membrane groups showed lower contact angle compared to Blood/ membrane groups ($p < 0.05$) [Welch and Brown-Forsythe followed by the Games-Howell's].

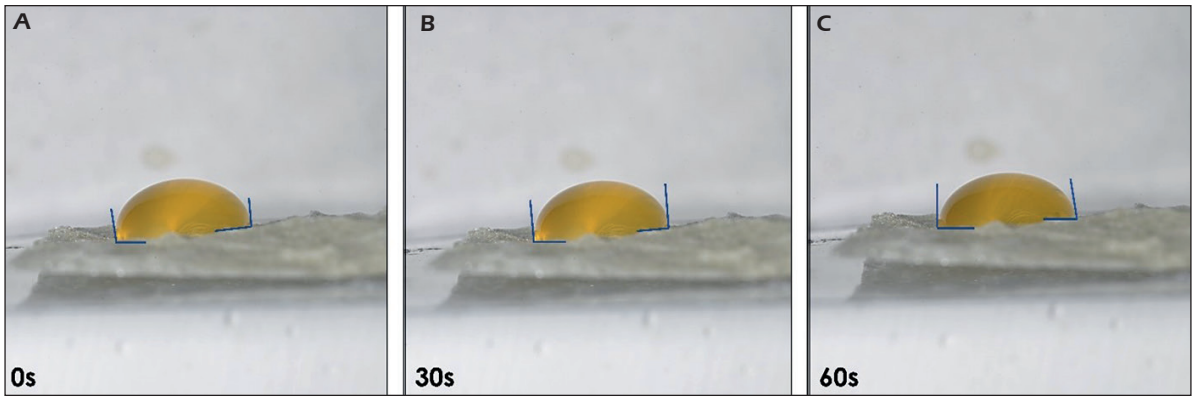

Figure 5. Detail of the group I contact angles at t0 (**A**), after 30s (**B**) and 1 minute (**C**).

30s and 3.31% after 30s (p<0.05). Conversely, the APL/2 mm membrane group reported a mean variation of the 1.05% (p>0.05) after 30s and 3.70% after 60s (p<0.05) (Figures 5-7).

Blood/Gel Mgha CA Membrane Timepoint Variations

The Blood/1 mm membrane showed a mean percentage variation of the 1.54% (p>0.05) after 30s and 4.56% after 1 minute (p<0.05). The group II of Blood/2 mm membrane showed an average variation of 0.89% after 30s (p>0.05) and 1.15% after 30s (p>0.05). Conversely, the Blood/3 mm membrane group reported a mean variation of the 0.12% (p>0.05) after 30s and 0.95% after 60s (p>0.05) (Figures 8-10).

Discussion

The physical and mechanical properties of the biomaterials and bone substitutes represents important key-points for the blood clot stabilization and the effective sustain of the osseointegration events[13,14]. In fact, the scaffold's affinity to the body fluids is able to produce a congruous fibro-cellular blastema and new bone formation, which conceptually represent the basic principles for a large part of the bone regenerative procedures in maxillofacial and orthopedics atrophies[15-17]. The main findings of the present investigation revealed a significant difference in the contact angle between the APL/membrane and Blood membrane for all thicknesses subgroups (p<0.05). In addition, the MG/Ha membrane thicknesses seems to produce no significant differences on contact angles measurement for both APL and blood groups (p>0.05). The wetting and adsorption of the fluid drops are determined by the biomaterial surface porosity, the scaffold surface tension, and fluid surface tension. In literature is still debated whether the blood surface tension represent a fluctuant parameter potentially influenced by several donors' variables (e.g., age, gender, hematocrit) and environmental

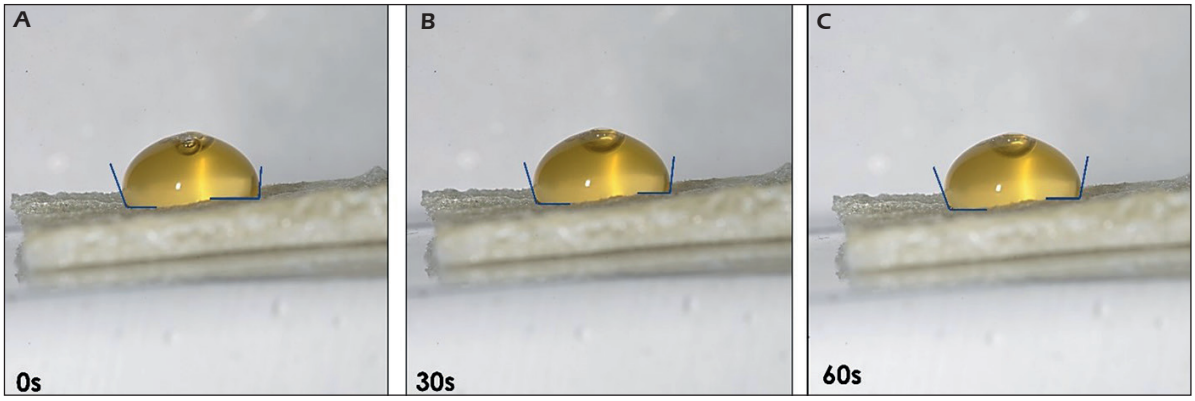

Figure 6. Detail of the group II contact angles at t0 (**A**), after 30s (**B**) and 1 minute (**C**).

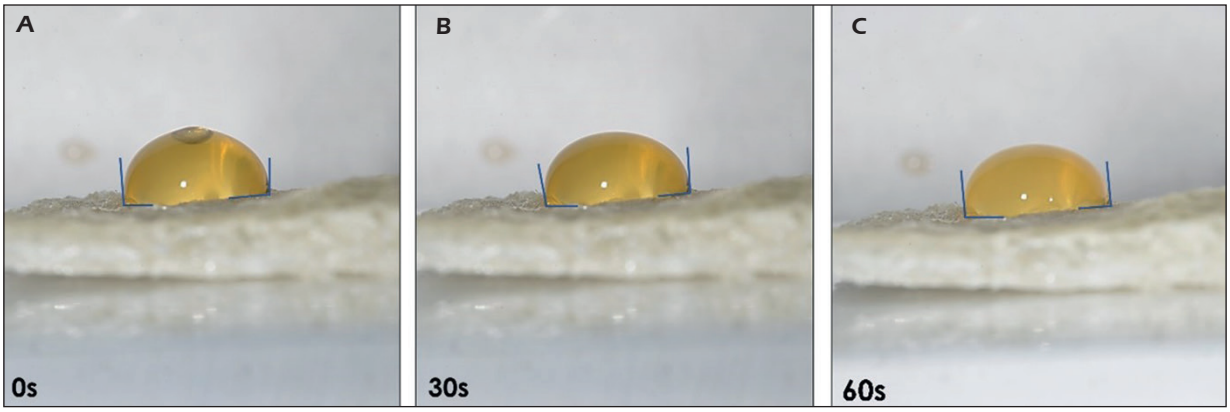

Figure 7. Detail of the group III contact angles at t0 (**A**), after 30s (**B**) and 1 minute (**C**).

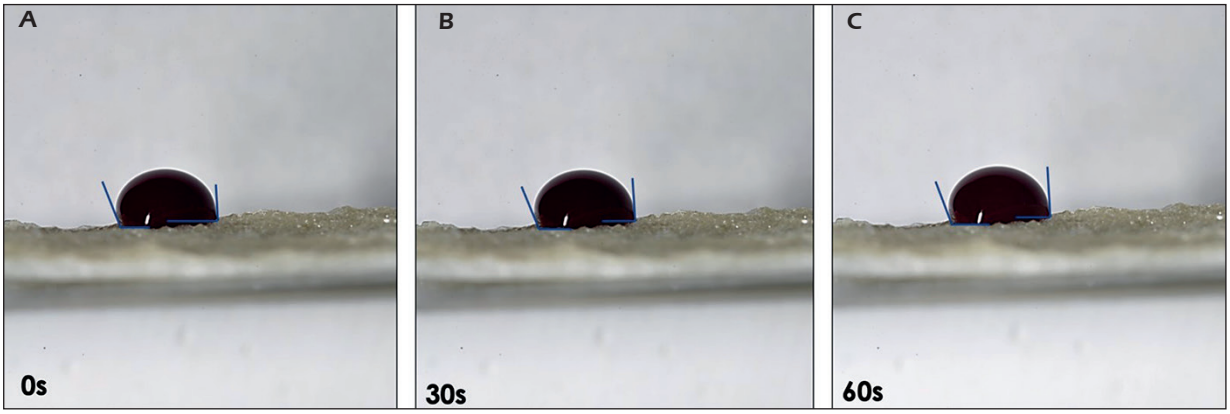

Figure 8. Detail of the group IV contact angles at t0 (**A**), after 30s (**B**) and 1 minute (**C**).

conditions (e.g., temperature, pH, humidity)[18-20]. Hrncír and Rosina[21] measured a blood surface tension of $55.89 \times 10^{-3} \pm 3.57 \times 10^{-3}$ N × m^{-1}, with no statistical correlation with red cell sedimentation rate, hemoglobin levels, erythrocytes, serum cholesterol and triacylglycerols, creatinine levels, ALT, and AST activity[21]. In literature, the blood serum plasma has been associated to a very wide range surface tension due to environmental temperature, donors' variables, and eventual pathological conditions[22,23]. In fact, Harkins[22] reported a blood serum surface ten-

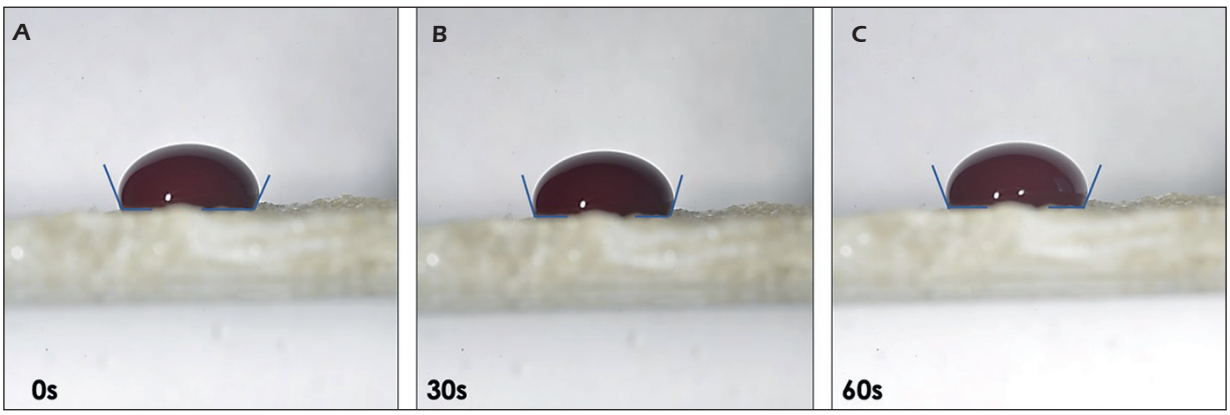

Figure 9. Detail of the group V contact angles at t0 (**A**), after 30s (**B**) and 1 minute (**C**).

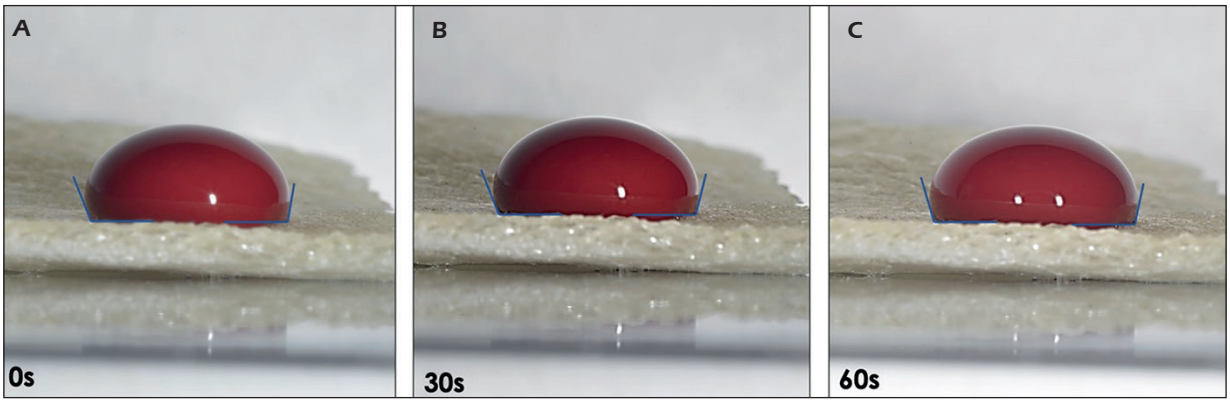

Figure 10. Detail of the group VI contact angles at t0 (**A**), after 30s (**B**) and 1 minute (**C**).

sion means range from 46.22 × 10^{-3} N × m^{-1} to 54.26 × 10^{-3} N × m^{-1}, respectively, at 10°C and 37°C. Consequently, it could be supposed that also the difference in centrifugation protocols as well as the eventual anticoagulant addition to platelet-derived emocomponents could play an important role on their viscoelastic properties. Clinically, the platelet-derived emocomponents and APL combined to the scaffold represent a versatile tool to obtain an increase of the mechanical behavior and plasticity of the graft, that is a remarkable advantage for the bone defect adaptation[13]. In addition, Scarano et al[13] reported that the autologous platelet liquid (APL) mixed with bovine bone particles is able to increase the Young elastic modulus by 117.2% compared to blood in combination with bovine bone particles[13]. Moreover, this combination could produce a biological advantage associated to the generation of a local reservoir of growth factor.

Conclusions

In conclusion, the static contact angles assessed with the sessile drop technique revealed similar wettability properties between APL and blood for both of MG/Ha gel membranes thicknesses tested. As expected, the MG/Ha gel membranes revealed a high hydrophilicity that could produce an increase in the reparative potential of the bone defect site, and an advantage in bone regeneration procedures.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Funding

This research received no external funding.

Authors' Contribution

Conceptualization, A. Scarano; methodology, A. Scarano, F. Lorusso; software, A. Scarano, F. Lorusso; validation A. Scarano, C. Mortellaro, Alberta Greco Lucchina; formal analysis A. Scarano, F. Lorusso; investigation, A. Scarano, F. Lorusso,; resources, A. Scarano; data curation, A. Scarano, F. Lorusso; writing-original draft preparation, A. Scarano; writing-review and editing, A. Scarano, F. Lorusso; visualization, A. Scarano, C. Mortellaro, Alberta Greco Lucchina, G. Falisi, C. Bugea; supervision, A. Scarano; project administration, A. Scarano; funding acquisition, A. Scarano. All authors have read and agreed to the published version of the manuscript.

Informed Consent

Written informed consent was obtained from the subjects involved in the present research.

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References

1) Kistler JO, Booth V, Bradshaw DJ, Wade WG. Bacterial Community Development in Experimental Gingivitis. Glogauer M, PLoS ONE 2013; 8: e 71227.
2) Palumbo A. The anatomy and physiology of the healthy periodontium. Gingival Diseases-Their Aetiology, Prevention and Treatment Croatia: InTech 2011; 1-21.

3) Bartold PM, Van Dyke TE. An appraisal of the role of specific bacteria in the initial pathogenesis of periodontitis. J Clin Periodontol 2019; 46: 6-11.
4) De Filippis A, Fiorentino M, Guida L, Annunziata M, Nastri L, Rizzo A. Vitamin D reduces the inflammatory response by Porphyromonas gingivalis infection by modulating human β-defensin-3 in human gingival epithelium and periodontal ligament cells. Int Immunopharmacol 2017; 47: 106-117.
5) Imatani T, Kato T, Okuda K. Production of inflammatory cytokines by human gingival fibroblasts stimulated by cell-surface preparations of Porphyromonas gingivalis. Oral Microbiol Immunol 2001; 16: 65-72.
6) Villar CC, Cochran DL. Regeneration of periodontal tissues: guided tissue regeneration. Dent Clin 2010; 54: 73-92.
7) Abdulghani S, Mitchell GR. Biomaterials for In Situ Tissue Regeneration: A Review. Biomolecules 2019; 9: 750.
8) Williams PM, Poh CF, Hovan AJ, Ng S, Rosin MP. Evaluation of a suspicious oral mucosal lesion. J Can Dent Assoc 2008; 74: 275-280.
9) Chaturvedi P, Majumder SK, Krishna H, Muttagi S, Gupta PK. Fluorescence spectroscopy for noninvasive early diagnosis of oral mucosal malignant and potentially malignant lesions. J Cancer Res Ther 2010; 6: 497–502.
10) Gynther GW, Rozell B, Heimdahl A. Direct oral microscopy and its value in diagnosing mucosal lesions: a pilot study. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2000; 90: 164-170.
11) Miron RJ, Pikos MA. Sinus Augmentation Using Platelet-Rich Fibrin With or Without a Bone Graft: What Is the Consensus? Compend Contin Educ Dent 2018; 39: 355-361.
12) Mozzati M, Martinasso G, Pol R, Polastri C, Cristiano A, Muzio G, Canuto R. The impact of plasma rich in growth factors on clinical and biological factors involved in healing processes after third molar extraction. J Biomed Mater Res A 2010; 95: 741-746.
13) Scarano A, Inchingolo F, Murmura G, Traini T, Piattelli A, Lorusso F. Three-Dimensional Architecture and Mechanical Properties of Bovine Bone Mixed with Autologous Platelet Liquid, Blood, or Physiological Water: An In Vitro Study. Int J Mol Sci 2018; 19: 1230.
14) Scarano A, Tari Rexhep S, Leo L, Lorusso F. Wettability of implant surfaces: Blood vs autologous platelet liquid (APL). J Mech Behav Biomed Mater 2022; 126: 104773.
15) Farré-Guasch E, Prins HJ, Overman JR, Ten Bruggenkate CM, Schulten EA, Helder MN, Klein-Nulend J. Human maxillary sinus floor elevation as a model for bone regeneration enabling the application of one-step surgical procedures. Tissue Eng Part B Rev 2013; 19: 69-82.
16) Jepsen S, Schwarz F, Cordaro L, Derks J, Hämmerle CHF, Heitz-Mayfield LJ, et al. Regeneration of alveolar ridge defects. Consensus report of group 4 of the 15th European Workshop on Periodontology on Bone Regeneration. J Clin Periodontol 2019; 21: 277-286.
17) Marx RE. Clinical application of bone biology to mandibular and maxillary reconstruction. Clin Plast Surg 1994; 21: 377-392.
18) Kratochvíl A, Hrncír E. Correlation between the blood surface tension and the activity of some enzymes. Physiol Res 2001; 50: 433-437.
19) Rosina J, Kvasnák E, Suta D, Kolárová H, Málek J, Krajci L. Temperature dependence of blood surface tension. Physiol Res 2007; 56: 93-98.
20) Yadav SS, Sikarwar BS, Ranjan P, Janardhanan R, Goyal A. Surface tension measurement of normal human blood samples by pendant drop method. J Med Eng Technol 2020; 44: 227-236.
21) Hrncír E, Rosina J. Surface tension of blood. Physiol Res 1997; 46: 319-321.
22) Harkins HN, Harkins WD. The Surface Tension Of Blood Serum, And The Determination Of The Surface Tension Of Biological Fluids. J Clin Invest 1929; 7: 263-281.
23) Zaitsev S. Dynamic surface tension measurements as general approach to the analysis of animal blood plasma and serum. Adv Colloid Interface Sci 2016; 235: 201-213.