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Synthesis of Extracellular Matrix by Airgel for Bone and Muscle Regeneration

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Abstract: This work was formed aerogels of polimetilmetacrilato, zinc and $CaCO_3$ (calcium carbonate) in presence of a polysaccharide matrix. The aerogels had a density about 6.33 and 116 mg/cm^3. These aerogels were put in muscle and bone tissue of laboratory Wistar rats, within the results obtained a satisfactory adaptation was observed in both tissues with no evidences of rejection that make sure to find a low cost route for the development of biocompatible aerogels to be used in the dental area and thereby effectuating the regeneration and repair of damaged tissues.

Key words: Aerogels, supercritical drying, extracellular matrix, PMMA, regenerate, $CaCO_3$.

1. Introduction

The development of biomaterials has had a huge progress in the entire world as they are a solution of many illnesses these might get involved organs or systems of the human body whether microscopic level or nanoscopic. One area to woken up the interest by the scientific world community is the production of airgels to different materials exhibited characteristics very specifically because they are compounds formed by a few percent of material that made and one great percent made up for air , 1% material versus 99% air [1].

However not all of them cannot have compatibility with living organisms by being composed heavy materials as the coal, copper, etc. The investigations have been focused to search alternatives with composed like the polysaccharides, proteins and amino acids that are constituent of source animal can be easily fit and recognized by the human body. These polysaccharides can use it as vehicle for transport medicines, electronic devices of side nanometric or serve like a counterfoil extracellular synthetic to repair and bony regeneration or muscular thanks to that they have a high porosity and they are extremely light [2].

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2. Planting of the Problem

Nowadays is a difficult problem the regenerate process on tissues specifics that have suffered damage at their structure to cause of accidents, pathologies, etc. For that reason, it is complicated to bring back the integrity because volume till one point the human body cannot regenerate tissues, then focuses on scarring as one alternative more effective to prevent infections from that giving us similar structure to the extracellular matrix we could develop aerogels to solve this problem of a simple form and low cost [3-6].

3. Objetives

The design of a supercritical drying chamber and the synthesis of polymethylmethacrylate airgels in the presence of a polysaccharide matrix for the creation of an extracellular matrix can serve as a growth and communication structure for any type of cells depending on the tissue that you want to regenerate [1, 7-12].

4. Theoretical Frame

4.1 Preparation of Airgels

The airgels are of the materials known lighter solids

from the man, they make up a polymer with a solvent that is a part of gel later with a process of supercritical drying and then will extract the solvent without the contraction so that it has the same initial volume. Are extremely porous and the low density this translucent material is one of the best thermal insulation available. Although the aerogels were invented in the decade of 1930, the NASA has been invented more invents inventors to create new types of airgels that are able to have a great impact in different scientific areas [3, 8].

5. Methodological Design

The following describes the steps followed for the preparation of the PMMA compound, for after the elaboration or the gel which will be subjected to subcritical and supercritical drying. Performing proofs in vivo gets results in the samples [12].

5.1 Formation of PMMA Compound, Zinc and Calcium Carbonate

This compound was created with the purpose to get an adaptation between woven bone and the airgels in order to just place it and without having to fix it or suture it in the area that we would like to regenerate.

Into a flask was placed 30 mL glacial acetic acid with 0.5 grams of zinc oxide and dropwise 2 mL of hydrochloric acid and stirred for 5 min until it had a homogeneous consistency. Subsequently 1 g of PMMA, 0.2 g of $CaCO_3$ and 10 mL of deionized water were added to form a gel.

In order to control the size of the particles, it was placed in a micro mill until it was powdered, once it was obtained, it was placed in a Erlenmeyer flask, 50 mL of deionized water, 0.59 gr of agarose and the material obtained from the micro mill, which we heated and mix with a magnetic stirrer and at a temperature of 37 degrees C for one minute, when dissolved it is placed in a Petri dish until it polymerizes and dries completely to collect the material scraping the surface of what was the gel, giving as a result spherical portions of the material that we synthesized previously.

In a Wistar rat, an incision was made in the portion of the thigh until the bone was visualized, with a carbide bur, an osteotomy was made to leave a concavity and the spherical particles that were previously synthesized were placed, the muscles were sutured and the skin with nylon thread and after one month the rat was sacrificed to observe the result in the lesion (Fig. 1).

5.2 Preparation of PMMA Airgel in the Presence of a Polysaccharide Matrix

Once created the material what helps us to adapt the aerogel in the bone , it continued with the develoment of the airgel.

We put into practice an Erlenmeyer flask with a magnetic stirrer with heater, thermometer and a Petri dish. A matrix of a polysaccharide with a concentration of 1% was used, 0.59 grams of the polysaccharide was placed in an Erlenmeyer flask, 50 mL of deionized water was added, after adding the PMMA compound, Zinc and $CaCO_3$, then stirred and it was heated at 37 °C on a magnetic stirrer until both compounds were homogenized for 1 minute, finally the solution was deposited in Petri dishes so that it was possible to polymerize and thus form the gel [1, 11], as shown in Figs. 2a-2d and proceed to the subcritical drying process.

5.2.1 Subcritical Drying

Subcritical drying is a procedure in which we replace the gel water in alcohol which is easier to extract by means of supercritical drying due to its boiling

Fig. 1 Bone with presence of compound PMMA/ZINC/$CaCO_3$.
Self-owned source.

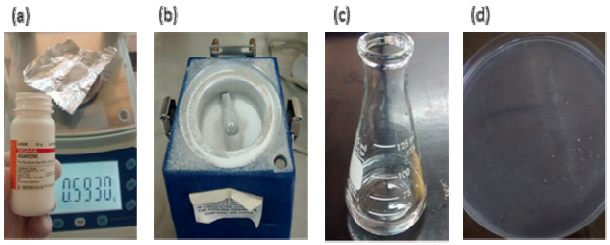


Fig. 2 PMMA gel formation.
Self-owned source.

point; the gel is left in 37 mL of 100% butane and 63 mL of 100% ethanol for 8 hours. Once finished it is changed again by 50 mL of butane and 50 mL of ethanol for 24 h. And finally it was left to rest 72 h in pure ethanol [11].

5.2.2 Manufacture of Supercritical Drying Chamber

With a 6" diameter inner tube and 4½" inside a car wheel on a lathe to leave regular surfaces, a ½" thick plate with a diameter of 6" was used for the formation of the rod. It was made bevel to weld it with solder 7,018 to form the upper plate also with a plate of 6" in diameter it faced and an interior step of 4½" was left as a guide when placing it [11].

He made 10 holes in the circumference along with the tube to be able to machiliate the holes to ⅜" standard. For the connections, a ¼" nipple was followed consecutively, followed by a T-shaped connection in which a high-pressure manometer and a high-pressure valve were placed on top to control the gas entering, for the release of the gas. Pressure was placed on the base of the chamber likewise a high-pressure valve [11].

5.2.3 Supercritical Drying

In this procedure the gel is placed in a supercritical drying chamber which supports more than 2,000 psi of pressure that is connected to a tank with a mixture of CO_2, argon and a heater. The gel is placed inside the chamber and pressurized up to 2,000 pounds critical point of CO_2 and heat the supercritical drying chamber between 60 and 65 degrees C for 12 hours for 3 days and two days thereafter was released per hour 100 pounds of pressure until you reach 0 [1, 11, 13].

5.3 Method for in vivo Testing of Airgel in Bone and Muscle

Once that had prepared the airgels it was proceeded to proof the same way in bone and muscle of Wistar rat , first an incision was made in the skin as straight and firm as possible, the access route was located according to the muscle fibers so as not to section the muscle transversally so that recovery was optimal. The folds were fastened with Allis forceps to have a good working field and avoiding damage to the soft tissues, osteotomy was performed in order to form a concavity and then airgel in the bone (Fig. 3a) once placed suture the muscle fold closest to the bone, then completely suture the muscle but place the airgel between the folds to observe the behavior in soft tissues (Fig. 3b).

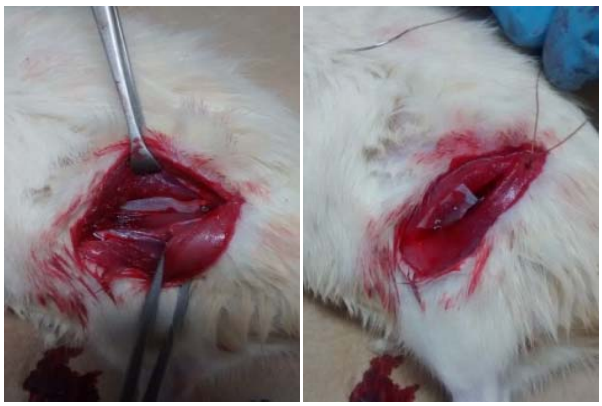
Finally, the skin is continually sutured for recovery.

6. Results and Discussion

An efficient method of manufacturing PMMA aerogels was obtained in the presence of a polysaccharide matrix with a mass of 0.0436 g which results in a density of 5.79 mg/cm^3 that is 0.21 times lighter than air (Fig. 4).

In the observation in a scanning microscope we can observe in the control gel that it was not processed by supercritical drying a surface.

Slightly irregular and smooth (Fig. 5) while in the gel subjected to supercritical drying spherical particles



A B

Fig. 3 Placement of airgel in bone and muscle.
Self-owned source.

Fig. 4 Macroscopic photo of the airgel.
Self-owned source.

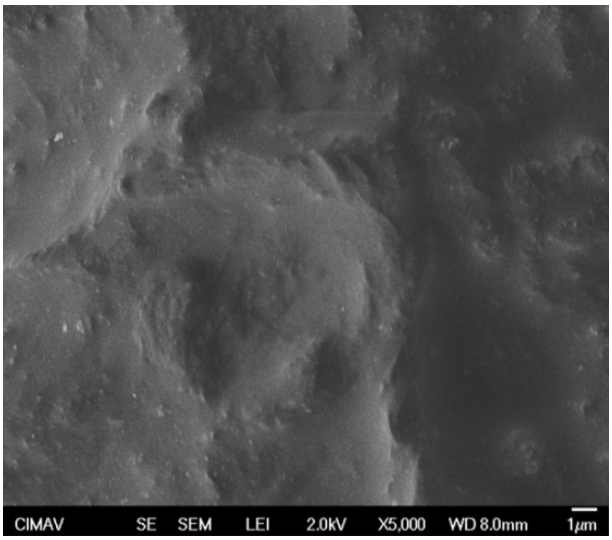


Fig. 5 Control gel without drying process.
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and porosity ranging from 50 to 200 nm can be observed as a result of which there was no contraction and replacement of the solvent by air (Figs. 6 and 7).

By means of TEM (transmission electron microscopy) (Fig. 8), we can observe a region of a particle that is a component of the airgel and that was deposited in the muscle of the rat, in this image the particle is attached to a muscle fiber, which means that the airgel has been favorably accepted in said fabric.

To observe the adaptation in the woven bone we took a radiograph to observe the material, due to the difference of density of this and the woven bone which one can observe in Fig. 9 in the concavity that formed the material was adapted to the surface creating a surface layer which joined without any means just placing it on the wound.

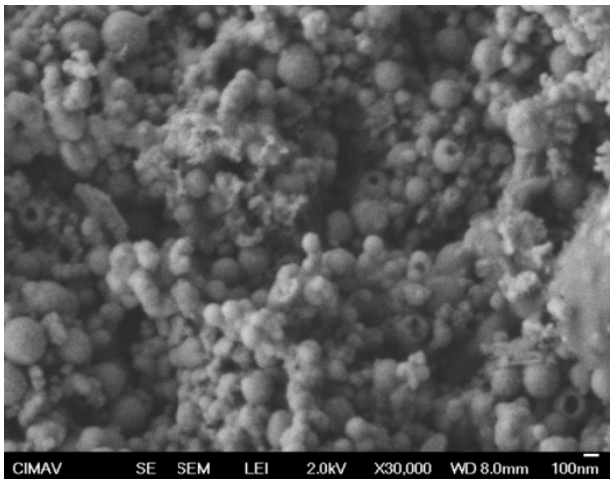

Fig. 6 Particles that make up the airgel in supercritical drying.
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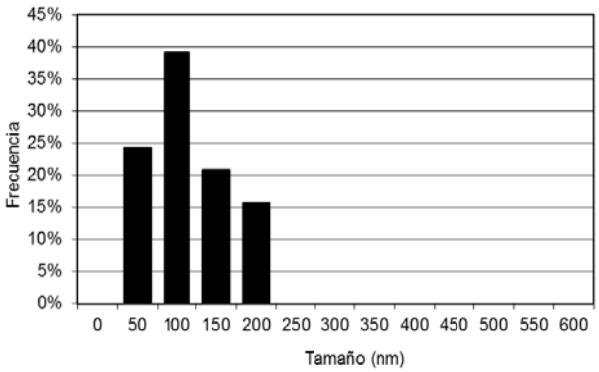

Fig. 7 Frequency distribution of airgel particles.
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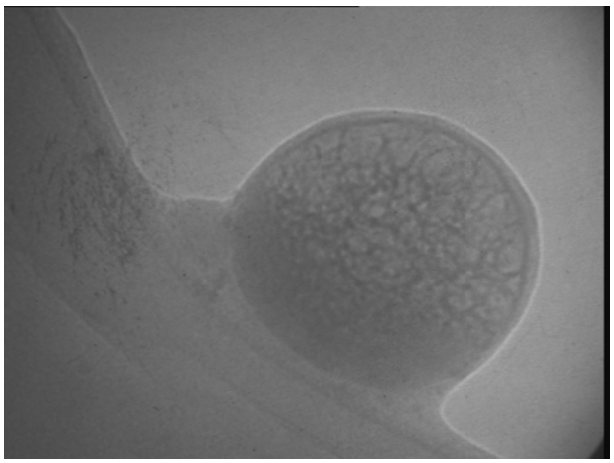

Fig. 8 Airgel constituent particle and its incorporation into rat muscle.
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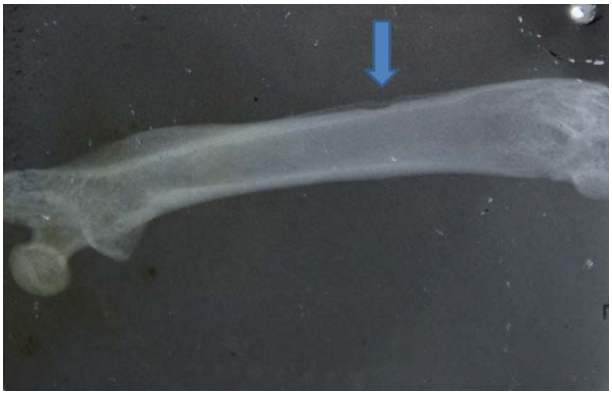

Fig. 9 Femur and airgel X-ray.
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7. Conclusion

All the results we got on this work show that is possible to get the elaboration of airgels through a low cost path, these micro and nanostructured materials can be used as models and can be applied in dental sciences, medical and tissue engineering research to replace or repair structures or damaged biological systems such as bones, muscle, tendons, nerves and in future complete organs of the body, they have unique characteristics such as great lightness and malleability.

References

[1] Meredith, J. E. Jr., Fazeli, B., and Schwartz, M. A. 1993. "The Extracellular Matrix as a Cell Survival Factor." *Mol Biol Cell.* 4 (9): 953-61.

[2] George, M., and Abraham, T. E. 2007. "pH Sensitive Alginate–Guar Gum Hydrogel for the Controlled Delivery of Protein Drugs." *International Journal of Pharmaceuticals* 335(1-2): 123-9.

[3] Klein, L. C. 1981. "Sol-gel Glass Technology. A Review." *Glass Ind.* 62: 14-7.

[4] Malafaya, P., Silva, G., and Reis, R. 2007. "Natural–Origin Polymers as Carriers and Scaffolds for Biomolecules and Cell Delivery in Tissue Engineering Applications." *Advanced Drug Delivery Reviews* 59 (4-5): 207-33.

[5] Ulrich, T. A., Juan Pardo, E. M. de and Kumar, S. 2009. "The Mechanical Rigidity of the Extracellular Matrix Regulates the Structure, Motility, and Proliferation of Glioma Cells." *Cancer Res.* 69 (10): 4167-74.

[6] Lu, P. F., Takai, K., Weaver, V. M., and Werb., Z. 2011. "Extracellular Matrix Degradation and Remodeling in Development and Disease." *Cold Spring Harb Perspect Biol.* 3 (12): a005058.

[7] Meredith, J. E. Jr., Fazeli, B., and Schwartz, M. A. 1993. "The Extracellular Matrix as a Cell Survival Factor." *Mol Biol Cell.* 4 (9): 953-61.

[8] Hough, L. A., Islam, M. F., Hammouda, B., Yodh, A. G., and Heiney, P. A. 2006. "Nano Lett, Structure of Semidilute Single-Wall Carbon Nanotube Suspensions and Gels." *Nano Lett.* 6 (2): 313.

[9] David, F. 2007. "Aerogelesmonolíticos de carbóncomoadsorbentes para la eliminación de compuestosorgánicosvolátiles (BTX), Quimicainorganica." *GEC.*: 1-5.

[10] Bryning, M. B., Milkie, D. E., Islam, M. F., Hough, L. A., Kikkawa, J. M., and Yodh, A. G. 2007. "Carbon Nanotube Aerogels." *Advanced Materials* 19: 661-4.

[11] Marquez, J., Carvajal, E., Miki, M., Alvarez, L., Rosa, A., Lizardi, J., and Rascón, A. 2013. "Water Extractable Arabinoxylan Aerogels Prepared by Supercritical CO_2 Drying." *Molecules* 18 (5): 5531-42.

[12] Paik, P., Mastai, Y., Kityk, I., Rakus, P., and Gedanken, A. 2012. "Synthesis of Amino Acid Block-Copolymer Imprinted Chiral Mesoporous Silica and Its Acoustically-Induced Optical Kerr Effects." *Journal of Solid State Chemistry* 192: 127-31.

[13] Fabrizioli, P., Bürgi, T., Burgener, M., Doorslaer, S., and Baiker, A. 2002. "Structural and Chemical Properties of Iron Oxide–Silica Aerogels." *Journal of Materials Chemistry* 12: 619-30.