Facial bones: fractures may be repaired with biomaterials made by using bacteria

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Bone MIMETISM

Cellulose and bioglass membranes stimulate cell regeneration

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Brazilian researchers are employing innovative approaches to develop biomaterials for medical and dental applications. These materials bind with cellular tissues and spur the formation of blood vessels, which results in faster bone recovery. One such bioactive material is a membrane made from cellulose produced by bacteria that includes laboratory-synthesized peptides (segments of proteins), in addition to bone elements such as collagen and hydroxyapatite, which can stimulate processes that improve bone repair. When in contact with physiological fluids, materials that are classified as bioactive, such as ceramics and glass, can regenerate the lost layer and bind with bone tissue. These materials are different from titanium, for example, which is often used to hold implants in place, but does not undergo effective chemical bonding with bone.

The composite material made from bacterial cellulose that was developed at Paulista State University (Unesp) at Araraquara (inner-state São Paulo) can be used for dental implants in cases where there is insufficient bone to hold the support pin or in cases where tooth extraction processes have resulted in bone shrinkage. Thus far, the trials conducted indicate possible applications to the repair of small bone fractures in body parts with low mechanical loading, such as in the facial bones. Cellulose is already commercially available and employed in medical applications, for instance, in antibacterial dressings that are used for treating burns; however, it has not yet been applied to the regeneration of bone tissue.

"We have introduced into the cellulose two types of peptides: one with five residues of amino acids and another with 14. The two fostered better bone repair," stated professor Reinaldo Marchetto from the Chemical Institute, who is the coordinator of the project and leader of a research group dedicated to the synthesis, structure and application of peptides and proteins at Unesp, Araraquara. Marchetto was the doctoral advisor to Sybele Saska, a dental surgeon who won an award at the 88th General Session of the International Association of Dental Research (July 2010, Barcelona, Spain) for her paper that was considered the best work in the Dental Materials category. The study is part of two projects financed by FAPESP and coordinated by Marchetto. Based on the results obtained, a patent application was filed with INPI, the National Institute of Industrial Property, with the aid of the Papi intellectual property support program, which is also run by FAPESP.

The cellulose is formed by nanofibers produced by bacteria of the *Gluconacetobacter* genus; each nanofiber has a length of approximately 10 to 50 nanometers; 1 nanometer is a millionth of a millimeter. The nanofibers are excreted by the bacteria when they are cultured in glucose, amino acids, yeast extract and salt for 120 hours at a temperature of 28°C. The layers of nanofibers are overlaid until they form a gelatinous-like blanket between the culture environment and the surface. When this material reaches a thickness of 5 mm, it is removed from the culture and washed to remove the bacteria. After undergoing a chemical treatment, cleaning with distilled water and sterilization, only pure cellulose remains, to which other components are added, such as collagen, hydroxyapatite and peptides.

After analyzing the physical and chemical properties of the material and performing resistance and traction mechanical trials, the researchers then conducted *in vitro* trials by cultivating bone precursor cells on the membranes with and without the addition of peptides for as long as 21 days. Marchetto noted that "the samples with peptides showed a far greater proliferation of osteoblast cells, which are young bone tissue cells, and the mineralization process was greater than that of the protein-free samples." The results suggested faster bone regeneration. The researchers then moved on to trials involving the guided bone regeneration of small defects in rat femurs. The analyses evaluated biocompatibility, efficiency of the regulating peptide, and bone density over periods of 7, 15, 30 and 120 days. According to Marchetto, "The peptide really spurred conduction and bone induction." After 15 to 30 days, new bone was formed. The initial trials indicated that reabsorption of the membranes by the body occurs over periods greater than 120 days. For the modified cellulose membrane to be employed in dentistry, however, further animal and human trials are required.

Bioglass produced at room temperature permits the addition of medical drugs with localized action

At the Federal University of Minas Gerais (UFMG), another biomaterial, i.e., a bioactive glass comprised essentially of silica, calcium and phosphorus, has been developed for bone regeneration in dental implants. This product may have future orthopedic applications, such as the repair of vertebrae or interaction with collagen. Its applications further extend to the replacement of bones with greater mechanical resistance, such as those in the legs and arms. Bioglasses manufactured by American companies are already present in the Brazilian market; however, the material developed at the university, which is being perfected at the startup firm Ceelbio in Belo Horizonte, is innovative because synthesis is conducted at room temperature. In addition to expending less energy, this process makes it possible to incorporate drugs into the product, with controlled release and localized action. "In the conventional process of melting the raw materials, followed by fast cooling, the bioglass is made at 800°C," according to professor Rosana Domingues from the UFMG Institute of Exact Sciences, who is the coordinator of the bioglass project and a partner in Ceelbio. "The high temperature makes the material dense and does not allow for the incorporation of medication."

The researchers chose a synthesis route called sol-gel formation, which consists of a sequence of chemical processes accelerated by a catalyst at room temperature. At the end of the process, a gel with a porous structure is obtained, which is then transformed into a powder to make it easier to prepare and to incorporate drugs. Trials for the evaluation of the material's toxicity that were registered with Anvisa, the Brazilian Sanitary Surveillance Agency, confirmed that it is non-toxic. At the Institute of Biological Sciences and the Dental School of UFMG, the researchers conducted trials *in vitro* and in rats, as well as preliminary trials in humans, using bioactive glass associated with antibiotics and anti-inflammatory drugs; these trials demonstrated favorable results.

The research at the university that led to the development of bioglass at room

Cellulose membrane after drying

THE PROJECTS

1. Synthetic peptides with application in the health field: perspectives for innovation and technological development – nº 2010/10168-8
2. Nanocomposites made from bacterial cellulose for use in bone tissue regeneration – nº 2009/09960-1
3. Nanocomposite materials made from bacterial cellulose, collagen, hydroxyapatite, growth peptides and related peptides, for use in bone tissue regeneration – nº 2009/50868-1

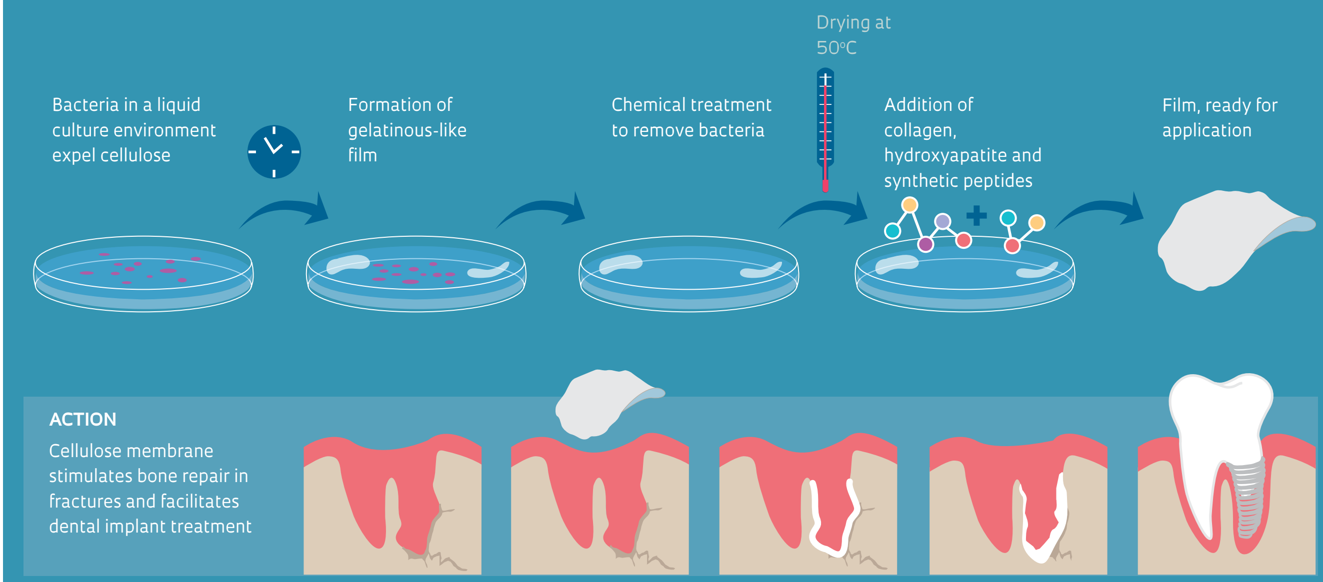
MODALITY
1. and 2. Regular Research Funding
3. Support Program for Intellectual Property

COORDINATOR
1., 2. and 3. Reinaldo Marchetto – Unesp

INVESTMENT
1. R$ 366,830.00 (FAPESP)
2. R$ 131,672.04 (FAPESP)
3. R$ 18,651.50 (FAPESP)

Bacterial cellulose

Step by step – production of the film for dental use



temperature began in the late 1990s with a doctoral student who, under the guidance of Rosana, created a biomaterial based on hydroxyapatite and zirconium. Since then, an exclusive research line has been established for the development of bioactive ceramic materials at UFMG's Department of Chemistry. The development of bioglass using the sol-gel synthesis route led to the filing of a patent application in 2002 and to studies on its commercial applications starting in 2008. In addition to Rosana, Professor Tulio Matencio, from the same department, is also a Ceelbio partner. At first, the firm was housed at Inova, which is the UFMG incubator for startups. However, because the incubator has no authorization to operate in the biological field, the company is moving to Habitat, which is the incubator operated by Biominas. Ceelbio works with ceramic materials along two different lines: one concerns fuel cells, i.e., devices similar to a hydrogen-based power generator, and the other concerns bioactive ceramics for biological applications. The project that led to bioglass received R$30 thousand in funding from Fapemig, the State of Minas Gerais Research Foundation, R$120 thousand from the Finep studies and projects finance agency and R$67 thousand for winning Desafio Brasil 2011, which is a prize for entrepreneurship and innovation granted by Intel and the Center of Studies into Private Equity and Venture Capital of the São Paulo School of Management of the Getulio Vargas Foundation.

Modification of the bioglass surface accelerates reactions that permit interaction with the organism

At the State University of Campinas (Unicamp), the research group led by Professor Celso Bertran from the Chemistry Institute functionally modified the surface of Bioglass 45S5, which is a commercial product composed of calcium, phosphorus, silicon and sodium. The functional modifications accelerate reactions that control the interaction of the bioglass with the organism, thereby inducing faster growth of bone tissues. "We modified the surface of the bioglass with calcium ions in a suitable concentration," stated Bertran, who is the doctoral thesis advisor of João Henrique Lopes, who has a FAPESP grant. This discovery resulted in Inova, which is Unicamp's Innovation Agency, filing a patent application with INPI. The modification accelerates the formation of calcium phosphate at the interface between the bioglass and bone tissue. "We were able to speed up the biological response of the bioglass without having to change the ease of processing," Bertran explained. "Both the process and the ensuing material are new."

Full characterization of the surface composition of the bioglass and determination of the speed at which the ions that comprise the modified surface are released into the tissue, thereby inducing bone formation and the binding of the bioglass with the host tissue, have already been conducted by the researchers. The original goal, to modify the surface of the bioglass while maintaining its vitreous properties, has been achieved. At present, the research is focused on determining the mechanisms of bioglass surface modification and on the biological evaluation of the material *in vitro*. ■

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