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To cite this version:
Sylvaine Jacquart, Agathe Belime, Christel Pigasse, Robin Siadous, Mohammed Fatnassi, et al.. Development of an injectable composite for bone regeneration. IRBM, Elsevier Masson, 2013, vol. 34, pp. 176-179. <10.1016/j.irbm.2013.01.008>. <hal-00869869>
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Eprints ID: 9371

To link to this article: DOI: 10.1016/j.irbm.2013.01.008
URL: [http://dx.doi.org/10.1016/j.irbm.2013.01.008]

To cite this version: Jacquart, Sylvaine and Belime, Agathe and Pigasse, Christel and Siadous, Robin and Fatnassi, Mohammed and Tadier, Solène and Auzély-Velty, Rachel and Girod-Fullana, Sophie and Bareille, Reine and Roques, Christine and El Kissi, Nadia and Anagnostou, Fani and Bignon, Aurélien and Cordier, Delphine and Rey, Christian and Brouillet, Fabien and Amédée, Joelle and Galliard, Hélène and Combes, Christèle. Development of an injectable composite for bone regeneration. (2013) IRBM, vol. 34 (n° 2). pp. 176-179. ISSN 1959-0318

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Development of an injectable composite for bone regeneration

S. Jacquet \textsuperscript{a}, A. Belime \textsuperscript{b}, C. Pigasse \textsuperscript{c}, R. Siadous \textsuperscript{d,e}, M. Fatnassi \textsuperscript{a}, S. Tadier \textsuperscript{a}, R. Auzély-Velty \textsuperscript{b}, S. Girod-Fullana \textsuperscript{f}, R. Bareille \textsuperscript{d,e}, C. Roques \textsuperscript{c}, N. El Kissi \textsuperscript{g}, F. Anagnostou \textsuperscript{h,i}, A. Bignon \textsuperscript{j}, D. Cordier \textsuperscript{k}, C. Rey \textsuperscript{a}, F. Brouillet \textsuperscript{f}, J. Amédée \textsuperscript{d,e}, H. Galliard \textsuperscript{g}, C. Combes \textsuperscript{a,*}

\textsuperscript{a} CIRIMAT, UPS-INPT-CNRS, ENSIACET, université de Toulouse, BP 44362, 4, allée Emile-Monso, 31030 Toulouse cedex 4, France
\textsuperscript{b} Centre de recherches sur les macromolécules végétales (CERMAV-CNRS), université Joseph-Fourier; 601, rue de la Chimie, 38041 Grenoble, France
\textsuperscript{c} LGC, UMR 5503 UPS-INPT-CNRS, faculté de pharmacie, université de Toulouse, 35, chemin des Maraîchers, 31062 Toulouse cedex 9, France
\textsuperscript{d} Inserm U1025, bio-ingénierie tissulaire, 146, rue Léo-Saignat, 33076 Bordeaux cedex, France
\textsuperscript{e} Université Bordeaux Segalen, 146, rue Léo-Saignat, 33076 Bordeaux cedex, France
\textsuperscript{f} CIRIMAT, UPS-INPT-CNRS, faculté de Pharmacie, université de Toulouse, 118, route de Narbonne, 31062 Toulouse cedex 4, France
\textsuperscript{g} Laboratoire rhéologie et procédés, domaine universitaire, 1301, rue de la Piscine, BP 53, 38041 Grenoble cedex 9, France
\textsuperscript{h} UMR CNRS 7052, laboatoire de bio-ingénierie et Biomécanique ostéoarticulaires, 10, avenue de Verdun, 75010 Paris, France
\textsuperscript{i} Service of odontology, Pitié-Salpêtrière hospital, Paris 7-Denis-Diderot University, UFR of Odontology, AP–HP, Paris
\textsuperscript{j} Medical Biomat, 5, chemin du Catupolan, 69120 Vaulx-en-Velin, France
\textsuperscript{k} Medical Lab, 5, chemin du Catupolan, 69120 Vaulx-en-Velin, France

Abstract

With the development of minimally invasive surgical techniques, there is a growing interest in the research and development of injectable biomaterials especially for orthopedic applications. In a view to enhance the overall surgery benefits for the patient, the BIOSINJECT project aims at preparing a new generation of mineral-organic composites for bone regeneration exhibiting bioactivity, therapeutic activity and easiness of use to broaden the application domains of the actual bone mineral cements and propose an alternative strategy with regard to their poor resorbability, injectability difficulties and risk of infection. First, a physical-chemical study demonstrated the feasibility of self-setting injectable composites associating calcium carbonate-calcium phosphate cement and polysaccharides (tailor-made or commercial polymer) in the presence or not of an antibacterial agent within the composite formulation. Then, bone cell response and antimicrobial activity of the composite have been evaluated in vitro. Finally, in order to evaluate resorption rate and bone tissue response an animal study has been performed and the histological analysis is still in progress. These multidisciplinary and complementary studies led to promising results in a view of the industrial development of such composite for dental and orthopaedic applications.

1. Introduction

As a result of ageing, of various life trauma/accidents and/or bone disease states, the number of bone fractures and trauma dramatically increases especially in the most industrialized countries where it raises a public healthcare issue. In this context, there is an increasing demand in the development of bone substitutes capable of being implanted using minimally invasive surgical techniques, offering rapid and effective bone repair at low cost, limiting postoperative infectious risk and enabling a fast transfer to industrial scale. In a view to enhance the overall surgery benefits for the patient, four main characteristics of the biomaterial have to be considered and investigated: injectability, resorbability, biological activity (especially osteogenecity) and antibacterial activity. The BIOSINJECT project proposes solutions to this multiconstraints problem and aims at preparing a new generation of mineral-organic composites exhibiting both bioactivity and therapeutic activity for bone regeneration at the best cost-efficiency ratio in a view to broaden the application domains of the actual bone mineral cements and propose an alternative strategy with regard to their poor resorbability.

This research program implements an inter- and multidisciplinary research based on a large consortium gathering eight national partners (six academic partners and two industrial
partners [small companies]). It is combining complementary expertises: material scientists for injectable composite material formulation, chemists to synthesise polymers with tailor-made properties, rheologists for rheological characterisation of composite paste, pharmacists for sustained release system formulation, microbiologists for antibacterial evaluation of the composite, cell biologists for cytocompatibility and biological activity evaluation of the composite in vitro, clinicians for the animal study and R&D engineers for production scale-up and technology transfer (Fig. 1).

2. Mineral and organic components

The matrix of the composite is based on calcium carbonate-calcium phosphate cements that have been studied and developed at CIRIMAT (Toulouse, France) during the last decade [1–3].

In addition, one of the main challenges of this project was to synthesise tailor-made polymers that can control paste cohesiveness and injectability suitable for mini-invasive surgery, drug release or biological activity. A patent has been filed on a process to prepare new biocompatible polysaccharide derivatives using click chemistry [4]. The feasibility of this new method was demonstrated in the synthesis of hyaluronic acid (HA) derivatives. In addition, functionalisation of a thermosensitive polymer and its grafting on HA backbone was also performed. Up to date, the amounts of chemically modified polymers synthesised using these two routes were not enough to fully evaluate the properties of the mineral-organic composites including these tailor-made polysaccharides. Consequently, even if these two routes of synthesis are still in progress, commercial polysaccharide additives have been chosen for the formulation of the paste used in this study.

3. Composite formulation and physical-chemical properties

Different ways of association of the polymer with the self-setting mineral powder were tested including polymer solution used as liquid phase to prepare the composite paste or polymer particles mixed with the mineral powder before preparing the composite paste using water as liquid phase. In the latter case, the polymer powder was used as received (commercial polymer powder) or was previously processed using spray drying to obtain microspheres with controlled particle size distribution which could be exploited to control the interconnected macroporosity of the cement on one hand, and to encapsulate biologically active agents on the other hand. The introduction of an antibacterial agent within the composite formulation was also investigated. Several proportions of polysaccharide and antibacterial agent were tested.

The injectability of the composite paste placed in a syringe was evaluated using a protocol previously published [5]. The setting chemical reaction was followed using complementary spectroscopic techniques (FTIR spectroscopy and solid state NMR spectrometry). An experimental procedure has been set and developed to finely analyse the composite paste rheological behaviour and the effect of the additives on the viscoelastic properties of the composite paste during setting using rheometry. The setting time was determined using the Gillmore needles standard method [6].
We showed that the introduction of the polysaccharide and of the antibacterial agent does not prevent the setting reaction to occur in the tested ranges. In addition the introduction of a polysaccharide improves the injectability of the paste whatever the way it is introduced in the paste; it also significantly decreased or inhibited the filter pressing phenomenon to occur during paste extrusion.

4. Composite in vitro and in vivo evaluation

Several biological in vitro studies were performed in the presence of bacteria or cells in contact with the prepared composite to evaluate bone cell and antimicrobial activity. We evaluated the antibacterial efficiency of the composite with/without antimicrobial agent against the main species involved in bone infections e.g. *Staphylococcus aureus* and *Staphylococcus epidermidis* using reference strains (Institut Pasteur Collection, Paris, France) and clinical isolates. Another in vitro study was performed using monolayers of human osteoprogenitors cells maintained in culture in the presence of the composite. The efficiency of the composite with regard to bone cell activity was investigated by measuring cell viability (live/dead), proliferation (MTT analysis) and differentiation determined by RT-QPCR of osteoprogenitor cells in direct contact with the composite. The results of these tests allowed us to determine the minimal proportion of antibacterial agent to introduce in the composite formulation in order to be efficient against bacteria and non-cytotoxic with regard to osteoblast cells.

Finally an animal study was performed to evaluate the reference mineral cement (without polysaccharide and antibacterial agent) and the composite material (with polysaccharide and antibacterial agent). Cements were implanted in critical size defects drilled in lateral femoral condyles of rabbits. After 6 weeks post-implantation, bone neoformation and material resorption were evaluated using non-decalcified histology and histomorphometry. For both materials compositions tested, no foreign body reaction, inflammation or fibrous tissue formation were observed. The CaCO$_3$-CaP cement showed high resorption and osteoconductivity. Histological study is currently in progress for composite specimen and the results will be included in another paper that will be submitted within the next months.

5. Communications

Several oral and poster communications where the ANR is acknowledged have been presented to international and national conferences:

Congress on Rheology, August 5–10, 2012 – Lisbon, Portugal;
• C14. Staphylococci biofilm, Control for the development of an antibacterial composite for bone grafts (poster). C. Pigasse, C. Roques. Eurobiofilms 2011, 6–8 July 2011, Copenhagen (Denmark);

6. Patent


Staff recruited for BIOSINJECT project

Non-permanent staff has been recruited to work full-time on BIOSINJECT project:

• Jacquart Sylvaine, PhD student (36 months), Institut National Polytechnique de Toulouse – CIRIMAT, France, (partner 1);

• Fatnassi Mohamed, post-doctoral researcher (12 months), Institut National Polytechnique de Toulouse – CIRIMAT, France, (partner 1);
• Belime Agathe, PhD student (36 months) at Université Joseph-Fourier, Grenoble – CERMAV, France, (partner 2);
• Pigasse Christel, Engineer assistant (18 months), Laboratoire de Génie Chimique, Toulouse, France, (partner 4);
• Siadous Robin, Engineer assistant (9 months), Inserm U1026, Bordeaux, France, (partner 5);
• Post-doctoral researcher (10 months), to be recruited, Laboratoire biomécanique et biomatériaux ostéarticulaires, Paris, France, (partner 6).

Acknowledgements

The authors thank the Agence nationale de la recherche (ANR–TecSan 2009 program) for supporting this interdisciplinary research project (BIOSINJECT – ANR-09-TECS-004 project).

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