Project no.:	**026739**
Project acronym:	**ExAct ResoMat**
Project title:	**External Activation of Resorbable Materials**
Instrument:	***Integrated Project***
Thematic Priority:	***Nanotechnologies and nano-sciences, knowledge-based multifunctional materials and new production processes and devices***

Final Publishable Report

Period covered: from **1st October 2009** to **11th March 2010**
Date of preparation: 9th **November 2010**

Start date of project: **1st October 2006** Duration: **48 months**

Project coordinator name: **Prof.dr. Nico Verdonschot**
Project coordinator organization name: **Radboud University, Nijmegen, The Netherlands**

Project Execution

Bioresorbable materials are currently under development to be used for trauma treatment, facial reconstruction, fracture treatment and bone defect reconstruction. Historically, most of these devices were made of metal and required a second operation to remove the device. This has led to the development of resorbable materials. The challenge with resorbable materials is to tune the resorption rate such that the material is being resorbed at the same rate the body is producing new tissue. The resorption rate can be controlled to some extend by material and geometrical parameters. However, in reality the resorption rate shows a large variation, depending on (local) patient characteristics. In addition, large variations occur in the rate of tissue repair amongst patients. For example, in some patients fracture healing occurs very slowly (diabetics, older patients). This illustrates the need for devices of which the resorption rate is not device-related, but can be tuned to the patient's needs and the response of the specific patient. Hence, this approach is radically different from current practice. The importance of the resorption properties limits the scope of materials that can be used for current applications. Resorbable materials can be used much more effectively if the physical rather than the resorption properties would be the decisive factors in the design phase.

The objective of the current project was to develop devices that can be implanted and which become resorbable only after externally emitted (from outside the body) stimulation. To reach the objectives, 8 state-of-the-art SME's and 3 research institutes, which all have a unique expertise in a specific field, have been carefully selected and integrated in this project.

Over the years substantial progress in various areas was made, but the technical project results regarding the External Activation Technology were far behind schedule. The biggest problems were related to the fact that we could not prove that capsules were perforated by ultrasound, we could not obtain a (bio)compatible combination of capsules in a coating, could not prove the activation of these capsules within the coating and could not define a coating that was impermeable against water for a relatively long period of time.
As a consequence a re-orientation of the project was required and the project objective was re-formulated at the beginning of the 4th year. We defined a new solution where we wanted to enclose capsules in a gel-like material and attach this capsule-containing-gel mixture in pockets in a device, the Hip Spacer produced by one of the industrial partners. The capsules should be disrupted with an external ultrasound signal, which should trigger release of antibiotics. To achieve this, a new project plan for the 4th year was written. However, due to the lack of progress the project was terminated on March 11, 2010.

Due to the lack of progress and difficulties in reaching the objectives the impact of the project on the orthopedic industry or research was minimal. There were a few milestones reached, however, they were not sufficient to allow the project to succeed.

Dissemination and use of the knowledge

Given that we were awaiting results about the proof of principle before making more steps towards dissemination outside the consortium, we produced only a few publications and presentations at conferences, but due to the lack of publishable results the dissemination activities were very low. However the academic partners have some papers in draft.

Project major achievements

PCL capsules with an additional crosslinkable monomer were prepared. By crosslinking the shell of the capsules using either UV light or heat, a better stability was obtained. The stability of the capsules was, however, insufficient to withstand neither dichloromethane nor even ethanol under shear. Therefore, the crosslinked PCL capsules do not lead to a sufficient increase in capsule solvent stability to be used in the final PCL coating (process).
To demonstrate that the capsules could be disrupted in a matrix they were embedded in ethyl cellulose (this was the demonstrator coating material). Micro-CT scans showed a good distribution of the capsules in the coating, which was encouraging, but it appeared that we could find no evidence that the capsules could subsequently be disrupted using ultrasound in a repeatable manner. However, the capsules had been disrupted by Echoson in a previous study, but this could not be repeated. Using SEM Echoson suggested that the capsules may not always be hollow.
To enhance the compatibility of the capsules with the PCL coating, PLLA capsules were fabricated by Encapson. New PLLA capsules were prepared with a hollow interior and tested for their activation using ultrasound. These tests were recorded using a high speed camera, however it was unclear whether the capsules react to ultrasound or whether cavitation occurs.
Echoson also tried to stimulate the PLLA capsules using ultrasound. Microcapsules suspended in single 1 mm layer of agarose gel were either destroyed by ultrasound or washed out by acoustical streaming. After addition of a second agarose layer, however all microcapsules remained unchanged, neither moved nor destroyed by ultrasound. Hence, a more powerful ultrasonic pressure was found required for which a new transducer needed to be build.
RUN tried to use a clinical Lithotriptor (kidney stone crusher) to damage a PCL coating on PMMA specimens. The tests were unsuccessful as the polymeric coating appeared to be insensitive to the shockwaves. The inclusion of Ca-P within the coating may increase its sensitivity to lithotriptic shock waves.
WUT investigated the method of using magnetic nano-particles (diameter about 10 nano-metres) in an alternating-current magnetic field. Various materials were considered and temperature increase was measured. The temperature increase was not enough to enable sufficient softening of the coating (e.g. PCL) material.
Gentamycin (antibiotics) filled PCL capsules could be prepared by Encapson and the loading of gentamycin could be varied. These were tested by Tecres in order to assess the loading of the capsules after preparation. Subsequently whether the antibiotics would remain in the PCL capsules was investigated. The results indicated that the antibiotics may leave the capsule once submerged in saline solution. This was caused by the degradation of the PCL material.
To reduce the permeability of the coating, Ca-P whiskers were embedded in the coating. Theoretically, these whiskers could serve as a water barrier and make the composite coating more suitable for water-barrier. In contrast, studies performed by InnoCore and WUT showed that the whiskers would increase the permeability.
Biomatec applied Cytotoxicity Tests and Genotoxicity Tests on polycaprolactone capsules in order to evaluate the mutagenic potential of the test article. For both tests it was concluded that, under the conditions of these studies, the tests articles showed no evidence of cell lysis or toxicity greater than a response index of 0/0 and no mutagenic in the tested species.
A very important study was performed to demonstrate the water barrier properties of PCL and PHBV (this material was potentially a better water barrier, but needs enzymatic degradation) on a 50/50 P(DL)LGA pin from Degradable Solutions. Both coating materials appeared not to be water-resistant enough to protect the core material from degradation. Furthermore, the experiment revealed some new insights. Coating a device may result in a more risky and

unpredictable product. Although there was room for improvement with respect to enhancing the water barrier the consortium agreed that it would be very difficult to find an appropriate coating that had adequate water barrier capacities.
With respect to WP3, Degradable Solutions has been working on the development of spinal cages containing Ca-P to make the cages stronger, stiffer and more biocompatible. A mold has been designed to allow for fabrication using a hot press process. Extensive time has been invested into the appropriate setting up of compression molding machine. So far Degradable Solutions has been able to compound of medical grade PLLA and β-Tricalcium phosphate (Ph. Eur. Grade, >96 % pure) in the ratio 70/30 % v/v. RUN developed a finite element (computer) model that could be used to optimize the cage design and simulate the consequences of cage placement.
Glasgow University fabricated various compositions of fibre-reinforced PLLA material and optimized the manufacturing settings (temperature, holding time, pressure). The produced samples were tested in 4 point bending (Flexural Strength and Flexural modulus were measured). Although the stiffness was higher than the reference sample, the increase in flexural strength for the manufactured samples was insufficient for the proposed applications.
RUN tested the mechanical strength of 5 types of Hip Spacers supplied by Tecres. The Hip Spacer was one of the main products that will be adapted during the course of this project. The studies run for millions of loading cycles (in the order of a few weeks) and consequently progression of the tests was steady, but slow. The test results were promising as they showed a large mechanical safety factor against fatigue failure of the Hip Spacer in clinical use.

In terms of sterilization (WP12) WUT investigated the influence of γ-irradiation performed on properties of PCL (polycaprolactone), PLLA (poly L-lactide) and PHBV (poly(3-hydroxybutyric acid-co-3-hydroxyvaleric acid). Theoretically a very low temperature during sterilization should limit the negative consequences of sterilization. Therefore the temperature was taken as a variable (either 23ºC or -78ºC). The results showed relatively little effect on this large difference in temperature. Furthermore, during post-irradiation aging, morphology and rheology of the studied polymers were affected thus testing of their features with time ought to be controlled.
The preliminary results of sterilization by exposure to ethylene oxide have shown that EtO appeared to be an effective method in terms of sterility for the studied materials and it not induce changes of macroscopic properties Only minor changes of mechanical properties were observed. Long term post-sterilization effects were under investigation.

In the 4th year of the project, Echoson succeeded (December 2009) in disrupting PLLA capsules in an agarose gel. Furthermore, by using the experimental set-up in Twente, Encapson was successful in disrupting thin-shelled PFO-PLLA capsules in water. So this was a good step forwards relative to the project plan. Furthermore, during the last months of the project Innocore developed the plan to release the antibiotics, Degradable Solutions continued to work on the spinal cages, WUT focused on the new project plan and the sterilization issues, GU proceeded to develop and test PLA-fibre reinforced materials, Tecres worked on the new Spacers which were tested by RUN. Biomatech and Magnet Physic did not perform any significant research activity during the last period. Animal studies as indicated in the original plan were not initiated at all.

The conclusion of this project is that it is very important that a project does not depend on a particular technology that has not been developed yet. A strong contingency plan needs to be installed which allows the consortium to move forward even if a chosen technology fails to work within a reasonable amount of time.