PROJECT NO: FP6-032045

CONTRACT NO: COOP-CT-2006-032045

Deposition of **E**ncapsulated **N**etworks of **D**rugs to prevent **R**emedial **I**mplant **T**reatment **E**manating from deep bone infection

Co-operative Research (Craft)

Horizontal Research Activities Involving SMEs

Entire Period Activity Report - Month 0 to 28

Date of issue of this report: March 2009

Start Date: 1st September 2006 Duration: 28 Months

SME EXPLOITATION MANGER: Finsbury Development

***SME CONTRACTORS*:**

1 Finsbury Development
2 Teknimed
3 Brace GmbH
4 Hunt Developments
5 Medicoat AG

RTD PERFORMER CONTRACTORS:

6 PERA Innovation Limited
7 Gothenburg University
8 Biomatech

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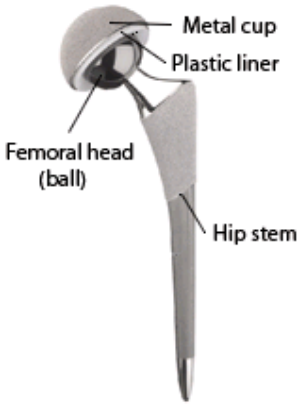
EXECUTIVE SUMMARY

This report covers the work carried out from Month 0 – Month 28 of the project. The main body of this report is, at the request of the industrial partners, an overview. However more detailed appendices are attached to cover the work programme, and the reports on specific task deliverables. The proposed CRAFT research project, **DENDRITE**, proposes to develop a surface coating for prosthesis implants such as hip and knee implants that will release drugs for the promotion and early fixation of the implants and for the prevention of infections. This will be done by the incorporation of a surface coating that will have both free form hydroxyapatite and polymer encapsulated antibiotic drugs and bone promotion agent that will be released over a time period.

The technical work over the first 6-month period (1st September 2006 – 31st August 2007) including the month 3 and month 6 meetings have been spread over the tasks in the following Work Packages. Work Package 1 – Characterisation of Drug Morphology, Work Package 2 – Synthesis of Novel Monomer / Polymer System, Work Package 3 – Encapsulation of Drug into Novel Polymer System & Work Package 4 – Coating Trials and Method Development.

Project Management, Co-ordination, Exploitation will be on going through the life of the project. The majority of tasks in Work Package 1 are now complete and good results have been achieved. Reports on each of these tasks have been attached to this document. Work on the initial tasks within Work Packages 2, 3 and 4 have also commenced. A kick-off meeting was held at the European Commission, Brussels, Belgium, on 20th September 2006. The project goals, work plan and initial actions were all successfully presented during the meeting and agreed. The month 3 management meeting was held at Brace GmbH, Karlstein, Germany on 24th January 2007. The month 6 management meeting was held at Medicoat AG, Mägenwil, Switzerland, on 25th April 2007. There have also been several technical meetings held in between the main management meetings. During the 12 months significant progress has been made on the scheduled work package.



The successful month 15/18 meeting was held at Finsbury, UK on 25th February 2008. The month 21 meeting was held in Amsterdam on 28th May 2008. The final project meeting (month 24) was hosted as a pan-European phone conference between all the partners. The technical work over the 2nd period (16 months) (1st September 2007 – 31st December 2008) including the months 15/18, month 21 and month 24 meetings have been spread over the tasks in the following Work Packages. Work Package 5 - Integration of the Encapsulation & Coating Technology, Work Package 6 – Innovation Related Activities, Work Package 7 – Consortium Management. All work activities are running on schedule. Project Management, Co-ordination, Exploitation will be on going through the life of the project. The tasks are now complete and good results have been achieved. Reports on each of these tasks have been attached to this document. There have also been several technical meetings held in between the main management meetings. During the 16 months significant progress has been made on the scheduled work package. The progress, against the package, is briefly outlined:

Work Package 1 - Characterisation of Drug Morphology.
This work package has been largely completed. A strategy has been developed to determine the most appropriate candidate drugs to use for this project.

Work Package 2 – Synthesis of Novel Monomer / Polymer System.
This work package has commenced. The designs and planning for the synthesis of the encapsulated drug with polymer have begun.

Work Package 3 – Encapsulation of Drug into Novel Polymer System.
This work package has commenced. The drug encapsulation has been accomplished *via* four different methods, where one will be taken forward for the final implant.

Work Package 4 – Coating Trials and Method Development.
This work package has commenced. The plasma coating of samples strips and animal model implants have begun.
Work Package 5 - Integration of the Encapsulation & Coating Technology.
This work package has been largely predominately completed. *In vitro* work has been successfully completed, *in vivo* work has commenced.

Work Package 6 – Innovation Related Activities.
This work package has been successfully completed. The production process of the drug encapsulated system has been investigated.

Work Package 7 – Consortium Management.
All consortium management tasks regarding the project have been completed.

A project web-page (http://dendrite.pera.com/) has been constructed so that all members of the consortium are up-to-date with progress in the project. It contains all relevant information for the project including; presentations, meeting minutes, meeting agendas and reports. The web-page is password protected and every member of the consortium has their own unique password to gain access into the portal. The site is regularly maintained and up-dated so it contains all the latest news and information.

1.0 PROJECTIVE OBJECTIVES & MAJOR ACHIEVEMENTS DURING THE REPORTING PERIOD

1.1 Overview of General Project Objectives

Objective and Targets

Our main objective is to develop a surface coating for prosthesis implants such as hip and knee implants that will release drugs for the promotion and early fixation of the implants and for the prevention of infections. This will be done by the incorporation of a surface coating that will have both free form hydroxyapatite and polymer encapsulated antibiotic drugs and bone promotion agent that will be released over a time period.

Economic Objectives to improve Competitiveness of a Large Community of SME's

By the end of year 5 after the project, we intend, through a network of trans-national and cross-sectorial licensees, to sell the encapsulation technology for the incorporation of drugs to both promote bone growth and provide antibiotic drug delivery over a pre-defined time period.

- Generate a new market sector which will compete with the USA drug manufacturers for antibiotics, displacing at lease 2.5% of the estimated €1.5 Bn p.a Global imported implants in to Europe and allowing the group of SME's to gain a footing into the Global market place by the development of the new process.
- Obtain for the SME partnership an additional 10% of the 1.4 million implant sales (140,000 additional implant sales) over the next ten years. We estimate that based on the average cost for the manufacture of Hip and Knee implants of €1,300 with an average profit of €325 we will increase the profit of the SMEs involved by €45.5 million per annum.
- The increase in turnover will have the effect of increasing employment within SMEs companies based on 1 person per €140,000 by (140,000 X 1300 = 182m / 140,000) an estimated 1300 jobs after the 10 year period.
- We also estimate that this coating will generate €116,000 million pa of healthcare cost savings due to the avoidance of complications in 140,000 patients from deep bone or soft tissues infections. However, we realise that this money will not be saved by the health care units but will actually allow them to reallocate this funding other areas of the health care sector, allowing these sectors to benefit from the additional funding.

Scientific objectives

Our scientific objectives are based around the surface treatment of the polymer encapsulation of a defined group of drugs for the delivery of antibiotics and bone promotion. We intend to:

- Enhance the understanding of processes that will allow the application of specific and intelligent coatings to orthopaedic implants. This has the benefit of:
- Identify the optimum encapsulation method for the encapsulation of a variety of drugs.
- Target therapeutic and prophylactic drugs to the site where they are needed.
- Determine the optimum combination of antibiotics, anti-inflammatory and bone promoting drugs, to fight infections and promote osteo-integration.
- Develop of the encapsulation process to allow for a targeted release of the drugs over a pre-determined time frame.
- Create a mechanically and chemically stable coating that will allow:-
    - Sterilization.
    - Adequate shelf life (currently 5 years).
    - Storage under a wide range of conditions to allow for global distribution.
    - Release of drug at correct rate.

This enhanced knowledge will be attained using the protocol described in work package 1 (B2) and will determine the encapsulation methodology, cross liking of the encapsulated drugs for timed delivery, coating adhesion to the substrate material and the sterilisation process required to facilitate the initial break down of the polymer encapsulation. It will also allow for the build up of several different coating layers.

Technological objectives

To develop a surface coating that will:

- Deliver between 0.1 to 10 mg of antibiotic (possibly gentamycin) and anti-inflammatories to the site of the infection.
- To obtain a timed release of the encapsulated drugs over a period of 1 hour to 3 years following implantation.
- To deliver 5 mg of bone promoting drug such a hydroxyapatite or calcium phosphate within the first 24 hours of insertion, to promote early fixation of the implant and increase the potential of osteo-integration with the surrounding bone tissue.
- Deliver an average of 2 mg of osteoblast promoting drugs per day for 8 weeks following surgery.
- To develop a process that will produce an even coating with a uniform thickness of 100 microns.
- To develop a suitable sterilization process that will not affect the encapsulation polymer or the drugs within.
- Obtain a coating material with a shelf life of 5 years following manufacture.

1.2 Summary of Recommendations from Previous Reviews

There are no recommendations from previous reviews.

1.3 Summary: Project Objectives & Achievements for Reporting Period 1 and 2

The specific objectives for the project from Month 13 to Month 28 are summarised in the table below:

Deliverable	WP	Objective	Progress Towards Achieving Objectives
D1	1	Drug candidate for the implant	The literature has been thoroughly examined to select appropriate drug candidates for this application.
D2	2	Plan synthesis method of the encapsulation polymer	Several synthetic strategies have been developed to encapsulate the drug with polymers.
D3	2	Prepare polymer for drug encapsulation	The preparation of the polymer with drug encapsulation has commenced.
D4	2	Selection of polymer for encapsulation	Several polymers have been successful for encapsulation and synthetic methods explored, investigation is still progress.
D5	3	Preparation of encapsulation strategy	Report on method giving functional products of known specification, investigation is still progress.
D6	4	Plan coating strategies	The coating strategies for the implants has been discussed and planned, investigation is still progress.
D7	4	Preparation for pre-clinical trials	Samples are currently being prepared for pre-clinical trials, investigation is still progress.
D8	5	Report on effects of selected encapsulated drugs and encapsulating materials on bone-promoting cells and monocytes	In vitro biocompatibility tests have been conducted and tests on osteoblast cells have successfully commenced using drug coated implants

Deliverable	WP	Objective	Progress Towards Achieving Objectives
D9	5	Report on the optimum combination of drugs and coating to promote early fixation and maintained long-term osteo-integration	In vitro biocompatibility tests have been conducted and in vivo tests on have successfully commenced using drug coated implants
D11	6	Show that prototype components have been designed and manufactured	Successful prototype implants have been designed and developed.
D12	6	Report on the standards, ethical and regulatory aspects of the exploitation of the results	Ethical and regulatory aspects of the exploitation have been addressed
D13	6	Industrial & Economic Validation	A full industrial and economic validation has been made on implant technology
D14	6	Produce a report on results to be published	A publishable summary has been completed
D15	6	Delivery of final report.	A final report has been successfully completed
D16	7	Delivery of a plan for the use and disseminating the knowledge the result obtained during the project.	A dissemination and use plan has been written and completed, explaining how results will be disseminated.
D17	7	Provision of audit certificates and bank guarantees and amended consortium agreement (if applicable)	Audit certificates have been provided by all consortium members.
D18	7	Report on gender, societal and ethical issues of exploitation.	A detailed report on gender, societal and ethical issues of exploitation has been constructed in line with the EC guidelines.

1.4 Problems / Issues during this Reporting Period

There have been no major issues or dire problems during the second reporting period. A four month extension was requested so that the work could be completed and reported fully.

2.0 WORK PACKAGE PROGRESS REVIEW FOR REPORTING PERIOD 1

2.1 Work Package Objectives

The specific work package objectives for the Period of Month 13 to Month 28 of the project are highlighted in bold in the table below.

Work-package No	Work package title	Lead contractor Short Name	Person-months	Start month	End month	Deliverable No
WP1	**Characterisation of Drug Morphology**	**Finsbury**	**18.2**	**0**	**8**	**D1**
WP2	**Synthesis of Novel Monomer / Polymer System**	**Brace**	**20**	**3**	**10**	**D2 D3 D4**
WP3	**Encapsulation of Drug into Novel Polymer System**	**Pera**	**15.7**	**8**	**12**	**D5**
WP4	**Coating Trials And Method Development**	**Medicoat**	**15.3**	**12**	**14**	**D6 D7**
WP5	**Integration of the Encapsulation and Coating Technology**	**Göteborg Uni.**	**10.3**	**14**	**28**	**D8 D9**
WP6	**Innovation Related Activities**	**Finsbury**	**12.5**	**0**	**28**	**D10 D11 D12 D13 D14**
WP7	**Consortium Management**	**Finsbury**	**3.4**	**0**	**28**	**D15 D16 D17 D18**
	TOTAL		95.4			

2.2. Overview of Work Package Technical Progress

Work-package 1: Characterisation of Drug Morphology

Task 1.1 Defining the Drug Component

Task Leader Finsbury

Objective: To arrive at a likely successful drug candidate, and therapeutic specification for use in the coating system.

Progress: Clinical and literature studies to determine which infections are most commonly found in both deep bone and soft tissue following surgery have been conducted. Gentamicin (antibiotic and bisphosphpnates (bone promoting drug) drug candidates have been identified to be employed to fight these infections including dosages currently administered. Further clinical research from literature and experts in the field have been carried out in order to determine the drug release profile appropriate to this orthopaedic application. Collation of all the data has been performed to identify the optimum drugs to use and the amount of drugs that are currently being delivered to the site of infection. Using the data from task 1.1.4 the optimum quantity of drug to be administered to fight the infection has been identified. This has involved a series of *In-vitro* tests using known bacteria and varying levels of antibiotic drugs.

Task 1.2 Test Drug Stability in Sterilisation Processes

Task Leader Hunt

Objective: To evaluate the selection of candidate drugs for their resistance to degradation in common sterilisation processes.

Progress: A series of tests with the monomer and polymer encapsulation material with regard to gamma irradiation sterilisation and potential timed degradation has been conducted. It was identified that ehe candidate drugs to be encapsulated will undergo the same testing as the monomer and polymer encapsulation materials outlined in task 1.2.1 to determine the optimum sterilisation process parameters. Analysis of the data generated has been used to determine the optimum drugs and dosages to be used and the possible encapsulation material.

Work Package 2: Synthesis of Novel Monomer /Polymer System

Task 2.1 Plan Synthetic Strategies for Novel Monomer/Polymer Systems

Task Leader Brace

Objective: Provide a method for the synthesis of a novel monomer and/or polymer which, bears phosphonyl moieties based upon a PLGA-type structure.

Progress: Monomers to those identified in WP1 and their methods of synthesis have been identified and analogous of polymeric materials for direct use have been identified. All information from tasks 2.1.1 and 2.1.2 has been collated and the most appropriate route for the manufacture of the monomer chains determined. Biocompatibility tests for the monomer/polymer to be used including a cytotoxicity culture test has been accomplished. These tests have been performed over various time periods and have been determined either qualitatively or quantitatively by examination of the cell morphology.

Task 2.2 Synthesis of Novel Phosphonyl Monomer and/or Polymer.

Task Leader Brace

Objective: To prepare novel monomer/polymer for use in the encapsulation of the drug candidates.

Progress: On the basis of 2.1.2 raw materials were obtained for each potential approach and attempt small scale reactions which will provide several small quantities of the desired monomer were conducted. From 2.2.1, basic methods have been established for making the desired monomers. Here the scale of the reaction was increased to furnish sufficient monomer to work with for the polymerisation reactions. Scale-up monomer synthesis, purification and characterisation followed by synthesis of larger quantities of monomer, have been conducted for the preparation of the next stage; that of exploring variants of polymer type and composition. A series of polymers have been used in different quantitie, so as to obtain a range of polymers. Quantities were sufficient to provide for follow up experimental work in encapsulation, and polymer characterisation.

Task Leader Pera

Objective: To fully examine the physical and chemical properties of the new polymer, for regulatory and practical purposes.

Progress: A range of techniques for the detection and examination of changes of either a chemical or physical nature on the polymer upon exposure to the sterilisation conditions were

accomplished. Size HPLC and zeta potential were useful in examining changes in molecular weight of the polymer. The polymer affinity for samples of solid substrate coated with hydroxyapatite was examined. The incorporation of bisphosphonate moieties had an attraction for HA surfaces. Surface topography studies were performed to examine this in great detail.

Task 2.4 Selection and finalising the polymer candidate(s) for Encapsulation Purposes.

Task Leader Finsbury

Objective: Deciding which of the polymers is (are) most suitable for incorporation into microcapsules and ensuring sufficient supply of product so chosen.

Progress: Information for the synthesis of the monomer/polymer and its use this in conjunction with WP1 to define optimum polymeric material for the encapsulation was conducted. This formed the basis for a report on the work performed to date and will give precise details of the polymer manufacturing process routes and type of polymeric material used. The information in task 2.4.1 was used to synthesise sufficient quantities of the chosen polymers. This was performed so that there is enough to perform the encapsulation process for the remainder of the project. The final programme of experiments determined the exact scale required, and an appropriate excess of the material desired was prepared and stored until needed.

Work Package 3: Encapsulation of Drug into Novel Polymer System

Task 3.1 Preparatory Operations: Encapsulation Strategy

Task Leader Pera

Objective: To review options available for the successful micro-encapsulation process, and to use judgement and experience to select best approach.

Progress: Detailed studied of the literature and commercial processes have been reviewed on what options are available in encapsulation technology. Specifically, this will include:

- high pressure water-in-oil-in-water emulsification,
- plasma spraying

The results of the literature survey will reveal methods that the best method was plasma spraying as the desired size and type of encapsulation could be achieved.

Task 3.2 Encapsulation Experimentation.

Task Leader Brace

Objective: To encapsulate candidate drugs with the novel polymer systems for characterisation

and preparation of bulk quantities allowing completion of the project.

Progress: Trial encapsulations using several methods, drug candidates and polymer types have successfully been conducted. Short practicals, experimentasl have been performed addressing each of the above variables constant whilst varying the others. This gave a combinatorial type of trial producing various products for further analysis. Application of all the promising approaches found from 3.2.1., were prepared of a range of combination microcapsule/polymer/drug combinations. Subsequent scale-up experiments were conducted.

Task 3.3 Complete Characterisation of Encapsulated Products obtained from Task 3.2.

Task Leader Pera

Objective: To fully examine the properties of drug-bearing microcapsules for practical purposes and for regulatory information.

Progress: Physical characterisation of microcapsules loaded with drug candidates were conducted:

- particle size and distribution analysis of the microcapsules,
- drug elution of the microcapsules

Measurements of the affinity of microcapsules to the HA surface models were investigated. Analysis of the drug release profiles, with and without prior gamma irradiation doses were investigated by measuring the rate of drug release from microcapsule. Concentrations of drug was measured in solution.

Work-package 4 – Coating Trials and Method Development

Task 4.1 Plan Strategy for Coating Layers

Task Leader Medicoat

Objective: To arrive at a practical plan for the coating approach.

Progress: All possible alternative approaches and methods for coating of the microcapsules in terms of conditions, techniques and concentrations were investigated. This was achived by literature searches the application of ideas both original and initiated from the literature. In particular plasma spraying was investigated. The most appropriate strategy was selected. Appropriateness criteria was efficiency of coating, quality of the finished coated layer and degree of technical difficulty

Task 4.2 Initial Primary Coating of HA Surfaces, with Microcapsules.

Task Leader Teknimed

Objective: Establish the basis for the coating methodology and provide feedback to the quality of that coating to enable positive responses.

Progress: Performance of trial coatings by binding to the HA surface of model substrate have been conducted. Several conditions and candidate microcapsule preparations have been applied. Partial characterisation of the coated surfaces providing feedback for further improvement of basic coating approaches was accomplished. Microscopy and surface roughness tests were conducted to investigate the surface topography. A layer of HA was coated on the substrate surface successfully. Dissolution tests were performed on candidate coatings to evaluate the dosage release rates for each coating process. The data from this was used for final coatings and tests for cytotoxcity.

Task 4.3 Preparation for Pre-Clinical Trials

Task Leader Göteborg University

Objective: To select candidate system(s) for progression into pre-clinical trials, and to ensure sufficient materials are available for those trials.

Progress: The coating work was reviewed and key model candidates were selected to be taken forward. Scientific judgements and decisions were exercised to select the gentamicin coated candidate for further study in pre-clinical trials. The controlled preparation of coated billet samples were performed and these were these samples were submitted for pre-clinical trials.

Work Package 5: Integration of the Encapsulation and Coating Technology

Task 5.1 *In vitro* effects on bone-forming cells of the combination of encapsulated drugs and materials

Task Leader Göteborg University

Objective: To examine the combined effects of encapsulated drug components and polymer-hydroxyapatite coatings on osteoblasts *in vitro*

Progress: Osteoblasts have been cultured on polymer-hydroxyapatite coatings with and without incorporated gentamicin in order to decide on the selection of drug and dosage and optimal material properties for the subsequent pre-clinical *in vivo* research. Human primary osteoblasts and osteoblast-like cell lines have been cultured on substrates consisting of different materials (coatings) selected and assembled in order to deliver encapsulated gentamicin over a time period

during which the response of cells are modified. Osteoblast viability and essential aspects of their functional repertoire (expression of alkaline phosphatase, expression of non-collagenous proteins and elaboration of extracellular bone-like matrix) have been determined to the material substrate, incorporated concentration of drug and culture time. Cytotoxicity tests have also been conducted and successfully performed on the drug candidates.

Task 5.2 *In vitro* effects on defense cells of the combination of encapsulated drugs and materials

Task Leader Göteborg University

Objective: To examine the combined effects of encapsulated drug components and polymer-hydroxyapatite coatings on monocytes *in vitro*

Progress: Monocytes have been cultured on polymer-hydroxyapatite coatings with and without incorporated gentamicin gentamicin in order to decide on the selection of drug and dosage and optimal material properties for the subsequent pre-clinical in vivo research. Human peripheral blood monocytes have been cultured on substrates consisting of different materials and coatings selected and these have been assembled in order to deliver encapsulated gentamicin over a time period during which the response of cells are modified. Monocyte viability and essential aspects of their phenotype and functional repertoire have been determined. Cytotoxicity tests have also been conducted and successfully performed on the drug candidates.

Task 5.3 *In vivo* effects on bone of the combination of encapsulated drugs and materials

Task Leader Göteborg University

Objective: Determine the optimum combination of bone-promoting, antibiotics and anti-inflammatory drugs on tissue response and osteo-integration of implanted material and coating

Progress:
Combinations of selected polymer and hydroxyapatite coatings and incorporated gentamicin have been prepared as test implants of different sizes and implanted in surgically created defects in the long bones of rabbits under sterile conditions. After different implantation periods, the implants and surrounding tissues have been excised *en bloc*, fixated, resin-embedded, sectioned and stained, this is currently being studied. The intact tissue-implant interface have been analysed qualitatively and quantitatively using light microscopy, histomorphometry and in selected cases using scanning electron microscopy (back scattered ion detection and element mapping) and transmission electron microscopy.

Work Package 6 : Innovation Related Activities

Task 6.1 Exploitation & Market Stimulation Plan

Task Leader Finsbury

Objective: To optimise the application of commercial resource among the partners, to develop and establish exploitation mechanisms.

Progress: An exploitation committee has been formed to plan the dissemination activities and exploitation routes**.** This has involved very thorough Patent searches to assess the viability of a patent application. Patent applications, have been successfully made, prepared and submitted through a patent agent. A draft Plan for the Use and Dissemination of the Knowledge has been created at the mid term and a final version at the final term. Formulation of an IPR ownership and exploitation agreement within the partnership and outside of the partnership has been discussed. Pre-marketing stimulation activities such as editorials, conference papers and planning for exhibitions, technology stimulation events and road shows are in progress for the very near future.

Task 6.2 Case Study Component Production

Task Leader Pera

Objective: A case study of the production process for the manufacture of the drug encapsulated system will be performed to demonstrate the overall concept is a practical, repeatable manufacturing process.

Progress: The process and production parameters have been evaluated to ensure that a repeatable component production is achievable. Production of the coated prototypes has been manufactured. Case study demonstrations have begun to assimilate the development technology.

Task 6.3 Design Guide Production

Task Leader Finsbury

Objective: Preparation and construction of best practice design and process guide.

Progress: Best practice advice and information from the project technical reports including design, testing results (mechanical & clinical), production and commercial details (contact details & relevant patents) has been discussed at project meetings. A generic design guide for the product design, manufacturing process operation and control has been developed to allow the partners to quickly absorb and apply the developed technology. Product publications in the form of editorials, technical papers and trade press are in progress for the coming year. Major exhibitions (MEDTEC, MEDICA, MDT) in the form of conferences have been attended.

Task 6.4 Industrial & Economic Validation

Task Leader Finsbury

Objective: Generation of complete cost analysis of the new manufacturing process.

Progress: The production and anticipated life cycle costs of the product have been investigated and provide an economic case for the use of the new implant. Partners have discussed to identify possible other applications of the processes developed and market areas for the product. Activities have been carried out to promote the early application of the results. It is envisaged that promising but not fully established technologies will be assessed, tested and validated.

Work Package 7: Consortium Management

Task 7.1 Knowledge management and innovation related activities

Task Leader Finsbury

Objective: To ensure that the knowledge management processes are conceived and implemented in a coherent manner.

Progress: A draft plan for the use and dissemination of the knowledge has been created and managed to ensure that a draft plan is made by the project at the final term. All individual tasks have been co-ordinated with each other utilising feedback loops that ensure they individually enable and complement each other. The oo-ordination of knowledge protection and modifications to IPR agreements have been assessed and made.

Task 7.2 Co-ordination of technical activities at the consortium level

Task Leader Finsbury

Objective: To ensure that all aspects of the EC requirements for communication and reporting are met.

Progress: The following technical activities have been accomplished:-

- Collation of all deliverables and milestone reports submitted to the EC and other partners.
- Submission of the cost statements and consortium agreements.
- Control of technical progress and ensuring that the project schedule is met.
- Review project progress against the economic, industrial and operational objectives and targets.
- Resolution of any administrative or contractual issues, including potential partnership instability.
- Organisation of Technical and Exploitation Board meetings.
- Provision of the minutes taken at these meetings.
- Technical risk contingency management.
- The partners will be required to attend technical project meetings.
- The partners will attend/hold relevant working party meetings.

Task 7.3 Co-ordination of legal aspects

Task Leader Finsbury

Objective: To co-ordinate the overall legal, contractual, financial and administrative management

of the consortium.

Progress: The overall legal, contractual, financial and administration management of the consortium have been coordinated. The consortium agreement between the participants has been updated and managed as necessary. Audit certificates have been obtained from each of the participants, as well as bank guarantees from the SMEs (if applicable).

Task 7.4 Co-ordination of other issues

Task Leader Finsbury

Objective: To co-ordinate gender equality, ethical and science and society aspects of the project.

Progress: The gender, equality, ethical, science and society issues have been coordinated. The Potential impact of the project has been identified and assessed related to the use of the results.

2.3 Deviation from the Plan and Corrective Actions

A four month extension was requested (and granted) by the EC, so that all remaining work could be completed on schedule. The project was extended to 31st December 2008. Appropriate changes in the Annex 1 were made to coincide with this. The total project duration was 28 months as a result.

2.4 Work Package Deliverables Update

Del. No.	Deliverable Name	WP No	Lead Participant	Estimated Persons Month	Nature	Dissemination Level	Delivery Month	Progress Towards Achieving Objectives	% Complete	Date Submitted to the EC
D1	Produce a report stating which drugs to use in the study and the doses/release profile required.	1	Finsbury	18.2	R	RE	3	The literature has been thoroughly examined to select appropriate drug candidates for this application.	100	April 2007
D2	Short technical note identifying the synthetic strategy	2	Brace	20	R	RE	6	The potential sterilisation processes for the implant and drug have been discussed and investigated.	100	April 2007
D3	Produce a short technical note describing the working basic method for synthesis of a single novel polymer	2	-	-	R	RE	7	Several synthetic strategies have been developed to encapsulate the drug with polymers.	100	August 2007
D4	Range of synthetic products/methods exploiting method from 2.2	2	-	-	P	RE	9	The preparation of the polymer with drug encapsulation has been completed successfully.	100	August 2007
D5	Report on method giving functional products of known specification	3	Pera	15.7	R	RE	12	A technical note describing the working method for synthesis of the encapsulated polymer has been described in detail.	100	March 2009
D6	Successful first coating of drug				P		14	Several polymers have been successful for	100	March

Del. No.	Deliverable Name	WP No	Lead Participant	Estimated Persons Month	Nature	Dissemination Level	Delivery Month	Progress Towards Achieving Objectives	% Complete	Date Submitted to the EC
	bearing microcapsules onto HA surfaces and multiple layering example	4	Medicoat	15.3		RE		encapsulation and synthetic methods explored.		2009
D7	Coated billets for pre-clinical trials	4	-	-	O	RE	14	Coated billets for pre-clinical trials have been made a coated with drug encapsulated polymers	100	March 2009
D8	Report on effects of selected encapsulated drugs and encapsulating materials on bone-promoting cells and monocytes	5	Götenburg University	10.3	R	RE	18	In vitro studies have been completed and in vivo studies have commenced.	95	March 2009
D9	Report on the optimum combination of drugs and coating to promote early fixation and maintained long-term osteo-integration	5	-	-	R	RE	21	In vitro studies have been completed and in vivo studies have commenced.	95	March 2009
D10	Exploitation plan	6	Finsbury	12.5	R	RE	12, 24	An exploitation plan has been set-up by the consortium members on the best methods to disseminate the results	100	March 2009
D11	Prototype components, including cost benefit analysis	6	-	-	P	RE	24	A prototype demonstrator of the drug coated implant has been developed, using different polymer and drug combinations.	100	March 2009

Del. No.	Deliverable Name	WP No	Lead Participant	Estimated Persons Month	Nature	Dissemination Level	Delivery Month	Progress Towards Achieving Objectives	% Complete	Date Submitted to the EC
D12	Design guide production	6	-	-	R	RE	24	A design guide production has been fully designed and developed.	100	March 2009
D13	Industrial & economic validation	6	-	-	O	RE	24	27 companies have been identified to be contacted directly to promote the project results.	100	March 2009
D14	Produce a report on results to be published	6	-	-	R	RE	24	A report on the results to be published has been accomplished	100	March 2009
D15	Delivery of six month progress report, midterm report and final report	7	Finsbury	3.4	R	RE	6,12, 24	All pending reports have been written providing details of the entire project.	100	March 2009
D16	Delivery of a plan for disseminating the knowledge	7	-	-	R	RE	12, 24	A plan for disseminating the knowledge at major events and in publications has been made	100	March 2009
D17	Provision of audit certificate and bank guarantees	7	-	-	O	RE	24	Audit certificates have been provided by partners	100	March 2009
D18	Report on gender, social and ethical issues of exploitation	7	-	-	R	RE	24	All gender, social and ethical issues have been investigated and thoroughly addressed.	100	March 2009
			TOTALS	**95.4**						

2.5 Work Package Milestones - Update

Milestone	Milestone Title	Completion Date	Update	Verification Level
M1	Presentation of clear specification for suitable drug candidates and their dosage/release profile required.	6	100 %	CO
M2	Presentation of a suitable synthetic strategy plan	9	100 %	CO
M3	A basic working method of synthesis	9	100 %	CO
M4	Presentation of a suitable synthetic strategic plan	12	100 %	CO
M5	Working prototype process.	12	100 %	RE
M6	Successfully coated bilayer sandwich array (HA-microcapsule-HA-microcapsule layer)	14	100 %	RE
M7	Data from relevant bone-forming cells and defense cells of their viability and temporal, functional expression after exposition to surface-coated material substrates/multilayer assemblies with differently incorporated drugs and dosages	24	100 %	RE
M8	Presentation of optimum combination of drugs and coating to promote osteointegration, thus serving as essential prerequisite for subsequent human clinical trials	24	100 %	RE
M9	Provide a report on the strategy and implementation for the translation of the project results into a protected form and provide a plan and timescales for patent protection of the new drug delivery system coating by month 18 of the project. This will be verified by discussion and agreement of all the partners that the plan and actions will secure the knowledge for commercial use and exploitation.	12	100 %	RE
M10	Transfer of the project knowledge from the RTD performers to the SME participants through the successful completion of 2 technology transfer events and interactions including 2 secondments and placements of 2 staff providing a total of 300 hours of technology transfer. Report on the critical knowledge elements to be transferred for successful exploitation by the SMEs verified by discussion and agreement of all the partners.	18	100 %	RE
M11	Completed promotion of the benefits of the developed technology and knowledge beyond the consortium to potential medical sectors including	24	100 %	R

	case study production, exploitation & industrial validation. Agreement by partners that the components produced satisfy the medical device directive (93/42/EEC) and (65/65/EEC).			
M12	Drug delivery system assessment and testing by third party users and experts.	24	100 %	P
M13	Demonstration to the EC and partners that all knowledge is created, managed and co-ordinated in a coherent manner. Verified by achievement of deliverables, effective reporting, and satisfaction of the contractual obligations to ensure that technical activities, legal aspects and other issues are co-ordinated.	12-24	100 %	R

R=Report RE=Restricted to Group Specified by the Consortium (inc. Commission Services)
P=Prototype, CO=Confidential, only for member of the Consortium (inc. Commission Services)

3.0 - CONSORTIUM MANAGEMENT

3.1 Consortium Status Overview

The consortium is working very well together providing valuable input and direction for the research programme. We have had a good start to the project with very constructive technical and commercial discussions at the meetings, as described in the minutes, and regular communication has taken place between the partners. The partners are working very well together, communicating and meeting regularly. In addition to the formal meetings a number of working party meetings have occurred to discuss the technical aspects of the project. The concluding project meeting went very well showing the strength and depth of the project results.

Project Management Structure

The project is controlled by a Technical Board, which in turn is headed by the Co-ordinator (Finsbury), who will have the ultimate responsibility for the project, and act as Chairman and Exploitation Manager. Each task has been allocated to the partner or RTD performer with the most appropriate skills or requirements relating to that particular task and they will be responsible for delivery of that task to plan. The task leaders are detailed in the work programme and report to the Co-ordinator.

3.2 Project Timetable & Status

3.2.1 Work Programme (Gantt Chart)

Work Schedule	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	Month
WP1 Characterise Drug																													9
1.1 Define drug component	x	x	x																										
1.2 Test drug stability in sterilisation				x	x	x	x	x	x																				
WP2 Synthesis of Polymer System																													10
2.1 Plan synthetic strategies				x																									
2.2 Synthesis of polymer					x	x	x	x																					
2.3 Characterisation on polymers									x	x																			
2.4 Selection for encapsulation										x																			
WP3 Encapsulation of Drug																													14
3.1 Preparatory operations									x	x																			
3.2 Encapsulation experimentation											x	x																	
3.3 Complete characterisation													x																
WP4 Coatings Trials																													18
4.1 Plan strategy for coating layers													x	x	x														
4.2 Primary coating of surface																x	x												
4.3 Preparation for pre-clinical trials																	x	x											
WP5 Integration of Technology																													28
5.1 In vitro effects of bone cells														x	x	x	x	x											
5.2 In vitro effects of defence cells																			x	x	x	x	x	x	x	x	x	x	
5.3 In vitro effects on bone & drugs														x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
WP6 Innovation Related Activities																													28
6.1 Exploitation & market plan	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
6.2 Case study component	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
6.3 Design guide production	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
6.4 Industrial & economic validation	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
WP7 Consortium Management																													28
7.1 Knowledge management	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	

3.2.2 Clarification of Changes to Work Programme

A four month extension was requested (and granted) by the EC, so that all remaining work could be completed on schedule. The project was extended to 31st December 2008. Appropriate changes in the Annex 1 were made to coincide with this. The total project duration was 28 months as a result.

3.3 Meetings & Communication

There have been nine meetings; including Management and Technical, since the start of the project.

	Date	Type of meeting		Location
1	20th September 2006	Kick-off	Management Meeting	European Commission
2	12th October 2006	Technical	Technical Meeting	Finsbury
3	30th October 2006	Technical	Technical Meeting	Brace
4	11th & 12th November 2006	Technical	Technical Meeting	Gothenburg University
5	24th January 2007	Month 3	Management Meeting	Brace
6	8th March 2007	Technical	Technical Meeting	Hunt Development
7	25th April 2007	Month 6	Management Meeting	Medicoat
8	31st May 2007	Technical	Technical Meeting	Pera
9	2nd August 2007	Technical	Technical Meeting	Finsbury
10	25th February 2008	Month 15/18	Management and Technical	Finsbury, UK
11	3rd April 2008	Technical	Technical	Pera, UK
12	9th May 2008	Technical	Technical	Finsbury, UK
13	28th May 2008	Month 21	Management and Technical	Amsterdam
14	19th June 208	Technical	Technical	Hunt, UK
15	26th June 2008	Technical	Technical	Brace, Germany
16	24th September 2008	Technical	Technical	Finsbury, UK
17	30th September 2008	Month 24	Management and Technical	Phone Conference
18	4th and 5th November	Technical	Technical	Finsbury, UK

3.4 Plans for Using & Disseminating Knowledge

Routes to Exploitation

A number of different types of Intellectual Property Rights (IPR) have been developed in this project. These include patents, designs (both registered and unregistered), copyright and (secret) know-how. The management of these different types of IPR has been allocated to the Exploitation Manager as part of the overall Exploitation Strategy (see below). Through consultation with Patent Attorneys and following close examination of the advice issued by the IPR-Helpdesk (e.g. 'A Tutorial On The Intellectual Property Regime of the Sixth Framework Programme' and 'The creation of an entity in charge of the exploitation of RTD results - What are the best choices?') and the sample consortium agreements supplied, the partners have produced a draft Consortium Agreement that comprehensively addresses the IPR issues in the Dendrite project.

The partnership has already developed an Exploitation Strategy for the management of knowledge, intellectual property and of its inter-relation with the various innovation-related activities planned. The basis of the strategy was to allow the new scientific knowledge created by the project to actively disseminated amongst academic communities to validate it and extend science and technology understanding and promote science and technology cohesion. However, prior to the programme of innovation related activities, the industrial partners have patented the technological capabilities developed and the product, process and system applications they in turn enable. Hence, the full range of scientific, technological and product, process and system specific dissemination, demonstration and training activities has been enableded without compromising the protection of the foreground IPR. This strategy included a joint policy of the partnership concerning rights of ownership, rights of defence and rights to exploit.

In summary, the SME participants in the Dendrite project are confident that they formed a strong supply chain, through its Exploitation Board was highly capable of the initial exploitation phase. Backed by strong project results leading to high quality and robust manufacturing and marketing processes, the participants were also confident that they met the requirements for international scale up of exploitation in new and diverse market sectors.

Assimilation and Exploitation of the results by the SME proposers

The members of the Dendrite consortium formed a close network of scientists, engineers and managers all working toward the common goals of the project. Technical meetings, design discussions, prototype manufacture, validation and trial work did all result in project personnel being seconded to multiple sites throughout the project. A fluent and convenient exchange of project reports and data has been achieved. All of these activities did promote rapid assimilation and retention of knowledge between the partners which continued beyond the end of the project. The SME partners formed an Exploitation Board and come together at specified meetings to co-ordinate and harmonise all the exploitation activities. This

committee generated an Exploitation Plan which linked companies, both in and outside the consortium, into an agreement that specify confidentially, licensing intentions and intellectual property protection. The Exploitation Board met on a frequent basis to review the EP, analyse market potential, sales forecasts and supply chain capability. The partners identified additional technology applications and market areas as well as disseminate the project results to a wider audience. After the project, the committee continued to plan and co-ordinate project developments and exploitation to ensure that the commercial collaboration and legal activities are continued and maintained.

Validation of the Technology

It is important that the product, process and applications are validated, and that market pull is stimulated to complement the technology push generated by the SME Proposers after the project. To assist in this, an application and technology demonstration facility will be produced to highlight the drug delivery system coating. Subsequently, validation trials of the DENDRITE coating system with both orthopaedic surgeons and professional/voluntary bodies will provide independent validation of our efforts. Additionally, periodic meetings of the Exploitation Board with members of the relevant regulatory approval organisation(s) will ensure the optimal route to approval is identified and followed. Additional meetings of the Board with trade associations will stimulate market exploitation and identify new and emerging applications for the technology.

Exploitation & Dissemination Activities Undertaken

From the start of the project, Pera established a website dedicated to the DENDRITE project. This had confidential and public domains. In the former all project reports, etc. were deposited with password protection so that only participants were able to gain access to it. In the latter was all the non-confidential material such as the project summary and news of project progress. Towards the second half of the project the public domain was used to disseminate all non-confidential project results. The site has been maintained for at least two years following the end of the project.

Through the website and through more conventional procedures such as publications in healthcare magazines, scientific journals and the popular media, organisation of workshops, seminars and conferences, the consortium encouraged the use of the DENDRITE technology in a broad range of medical, industrial and commercial applications. All participants played an active role in technology transfer and dissemination, promoting the technology development to customers, and through networks of industrial contacts. Where possible, links have been established with existing EC funded projects involving surgical

systems and technology. Trade Associations throughout Europe have been used to network the results and help demonstrate the technology to end users in a variety of industry sectors. The number of events, publications, etc. have been determined by the Exploitation Board in collaboration and was specified in WP7.

4.0 OTHER ISSUES

4.1 Conclusions

The project is developing correctly and initiating a number of novel technologies, which will unlock new market opportunities, for the consortium, and provide substantial social benefits. The consortium is working well together, steering the research partners, providing guidance, assistance and the specialist knowledge needed for a successful project. The successful month 15/18 meeting was held at Finsbury, UK on 25th February 2008. The month 21 meeting was held in Amsterdam on 28th May 2008. The final project meeting (month 24) was hosted as a pan-European phone conference between all the partners. The technical work over the 2nd period (16 months) (1st September 2007 – 31st December 2008) including the months 15/18, month 21 and month 24 meetings have been spread over the tasks in the following Work Packages. Work Package 5 - Integration of the Encapsulation & Coating Technology, Work Package 6 – Innovation Related Activities, Work Package 7 – Consortium Management. The planned tasks in each work package are progressing well and show very promising results. The technical research has progressed very well. Both Brace and Pera carried out the various methods for drug encapsulation. The five methods undertaken for drug encapsulation have all proven to be successful. The selected drugs for encapsulation are gentamicin and alendronate. These drug encapsulated microspheres and films have been tested for drug elution studies and fine results obtained. Prototype implants have been manufactured in the form of disks from titanium at Pera. Cobalt chrome disk and cylinder implants have also been manufactured. Stainless steel strips have also been made to study the surface topography after plasma coating with hydroxyapatite The TCP and HA has been provided by Teknimed for the plasma spraying on to cobalt-chrome or titanium. The plasma spraying of titanium and hydroxyapatite has been conducted by Medicoat.

The planned *in vitro* and *in vivo* work has commenced at Biomatech and Gothenburg University respectively. Biomatech have performed additional cytotoxicity tests for a more thorough check on the drug encapsulation. The results of this pre-clinical study would be written for publication. The paper would consist of the work conducted in the consortium collaboration.

The project has continued as envisaged; partner attendance has been excellent at all of the organised meetings. The consortium shows enthusiasm and has been highly motivated throughout the project duration. A patent application has also been made on the technology developed in the project.

APPENDIX

Deliverable 1

Summary

This report shows the required background research towards the research project, in identifying the diseases that need to be treated, the type of drug that needs to be used showing the release profiles and dosage information. It also shows potential methods for the sterilisation process. A thorough literature review was conducted on gentamicin being used as a drug in bone surgery and the extensive use of bisphosphonates.

Introduction

This report presents a thorough review of the literature to enable to identify the drugs to be selected for this study. It also shows from data in the literature a dosage and release profile to be used for the drug. It was discussed and agreed early on in the project that an antibiotic and a promoting agent would be required for the required type of orthopaedic implants. The literature has been researched on defining the drug component and the infections that need to be targeted. The Pub Med, Science Direct and Scopus databases have been researched to discover published papers and applications concerning the technology for the use of drugs in orthopaedic surgery. Enhanced knowledge has been gained on chemical approaches to the development of drug loaded-implants.

Firstly, the types of infection that the new technology had to combat were identified and described. The types of drug required to cure these diseases were then researched and addressed. From preliminary research on antibiotics, gentamicin was recognised as the antibiotic to be used for this study at an early stage (mentioned in the research proposal as the drug candidate), therefore was selected for this study.

Of the drugs for bone-promoting the bisphosphonate family were found to be the most prolific (from preliminary research mentioned in the research proposal as a drug candidate) and so are mentioned in this research.

The recommended drug doses and release profiles from literature reviews has also been mentioned.

The final section is on potential sterilisation methods to be used for this research.

Experimental

1 Types of Infection to be Targeted

The types of infection or bacteria that needed to be targeted for this drug delivery system;

1. Osteoporosis
2. Osteitis deformans (Paget's disease of bone)
3. Bone metastasis (with or without hypercalcemia)
4. Multiple myeloma

Osteoporosis is disease of bone in which the bone mineral density (BMD) is reduced, bone micro-architecture is disrupted and the amount and variety of non-collagenous proteins in bone is altered.

Figure 1 Osteoporosis of the hip

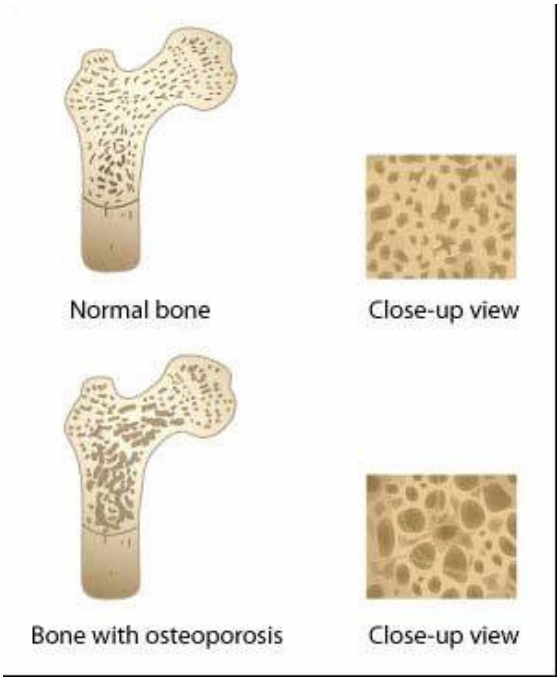
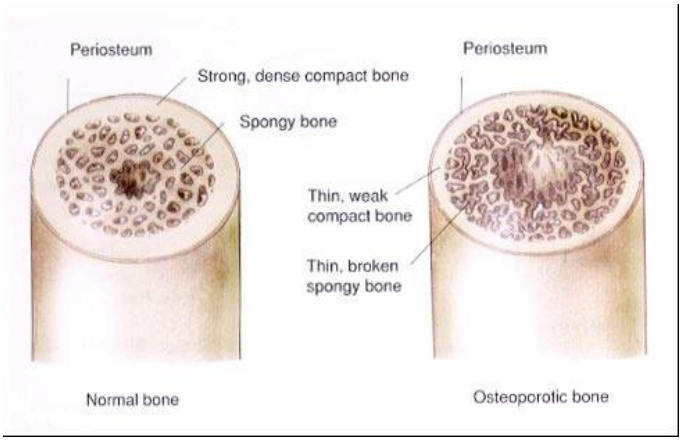


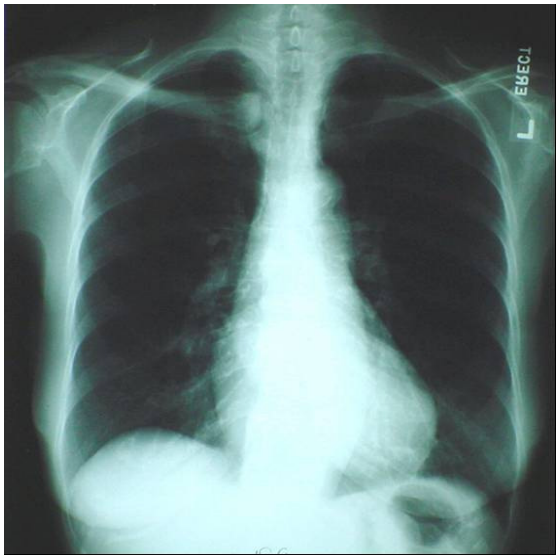
Figure 2 Osteoporotic bone



Osteitis deformans (Paget's disease of bone) is a chronic disorder which results in enlarged or deformed bones. Paget's disease causes an excessive breakdown of bone tissue, causing bones to weaken resulting in bone pain, arthritis, deformation and fractures.

Type of infection; Paget's disease may be caused by a slow virus infection. A slow virus is associated with a disease having a long incubation period of months to years with a gradual onset frequently terminating in severe illness and/or death.

Figure 3 Paget disease of the right clavicle, which is larger and markedly dense (sclerotic) compared to the left clavicle.

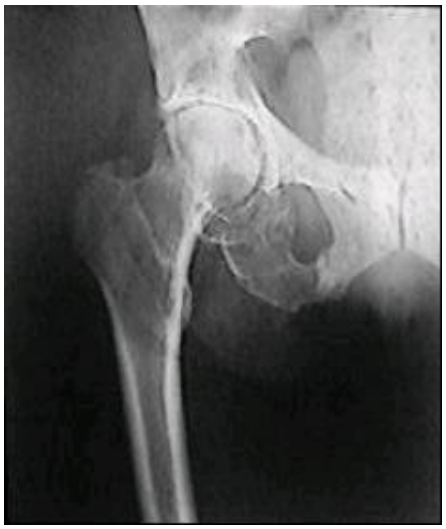


Paget disease of the bone (osteitis deformans) is a metabolic disorder characterized by abnormal osseous remodelling with increased resportion and bone formation. The newly formed bone is abnormally soft with a disorganized trabecular pattern.

It causes bony enlargement and occurs most commonly within the pelvis. Paget's disease has three distinct phases that are visible by x-ray (Figure 3).

Bone metastasis (with or without hypercalcemia) is the spread of cancer from its primary site to the bone. Hypercalcaemia is an elevated calcium level in the blood.

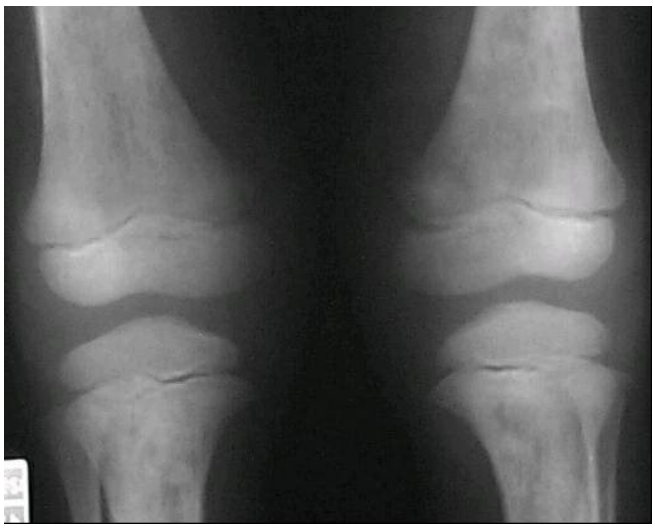
Figure 4 Bone metastasis of the hip



Bone metastasis is one of the most frequent causes of pain in people with cancer. It can also cause bones to break and high calcium levels in the blood (calcium is released from damaged bones). It also causes other symptoms and complications that can lower your ability to maintain your usual activities and lifestyle.

Cancer cells that break off from a primary tumour and enter the bloodstream can reach nearly all tissues of the body. Bones are one of the most common sites for these circulating cells to settle in and start growing. Metastases can occur in bones anywhere in the body, but they are mostly found in bones near the centre of the body.

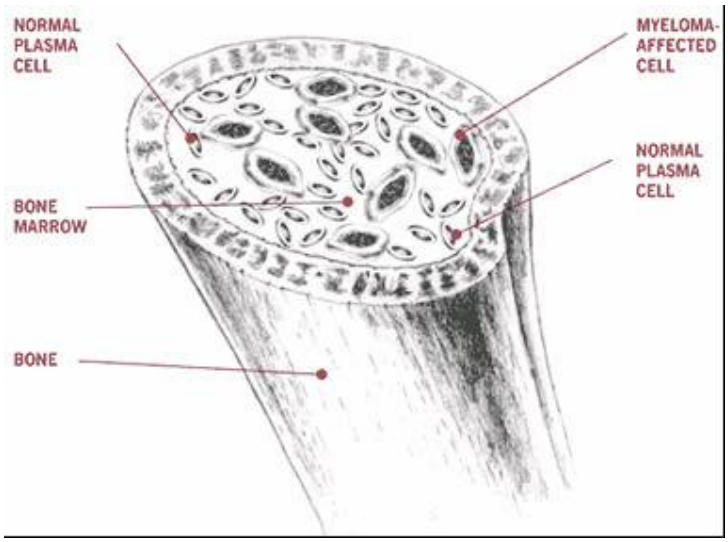
Figure 5 Bone metastasis of the knee



Bone metastases are not the same as cancers that start in the bone, which are called primary bone cancers. Bone metastasis and primary bone cancers are very different. Primary bone cancer is much less common than bone metastasis. Many people with cancer (except for those with non-melanoma skin cancer) may develop bone metastasis at some point in the course of their disease. The spine is the part of the skeleton most commonly affected by bone metastasis.

Multiple myeloma is a type of cancer of plasma cells, immune system cells in bone marrow that produce antibodies. Type of infection; Pneumonias and Pyelonephritis. Common pneumonia pathogens include *S pneumoniae*, *S aureus*, and *K pneumoniae*, while common pathogens causing pyelonephritis include *E coli* and other gram-negative organisms.

Figure 6 Bone myeloma



Multiple myeloma (also known as myeloma or plasma cell myeloma) is a progressive hematologic (blood) disease. It is a cancer of the plasma cell, an important part of the immune system that produces immunoglobulin's (antibodies) to help fight infection and disease. Multiple myeloma is characterised by excessive

numbers of abnormal plasma cells in the bone marrow and overproduction of intact monoclonal immunoglobulin (IgG, IgA, IgD, or IgE) or Bence-Jones protein (free monoclonal κ and λ light chains). Hypercalcemia, anaemia, renal damage, increased susceptibility to bacterial infection, and impaired productions of normal immunoglobulin are common clinical manifestations of multiple myeloma. It is often also characterized by diffuse osteoporosis, usually in the pelvis, spine, ribs, and skull.

2 Potential Drug Candidates for Orthopaedic Implants

The types of drug to target the for this drug delivery system;

1. Antibiotic – Gentamicin
2. Bone promoting agent - Bisphosphonate

Gentamicin

Gentamicin is an aminoglycoside antibiotic, and can treat many types of bacterial infections, particularly Gram-negative infection. However, gentamicin is not used for *Neisseria gonorrheae*, *Neisseria meningitidis* or *Legionella pneumophila* infections.

Gentamicin is a bactericidal antibiotic that works by binding the 30S subunit of the bacterial ribosome, interrupting protein synthesis.

Like all aminoglycosides, when gentamicin is given orally, it is not effective. This is because it is absorbed from the small intestine, and then travels through the portal vein to the liver, where it is inactivated. Therefore, it can only be given intravenously, intramuscularly or topically, *E. Coli* has shown some resistance to gentamicin, despite being Gram-negative.

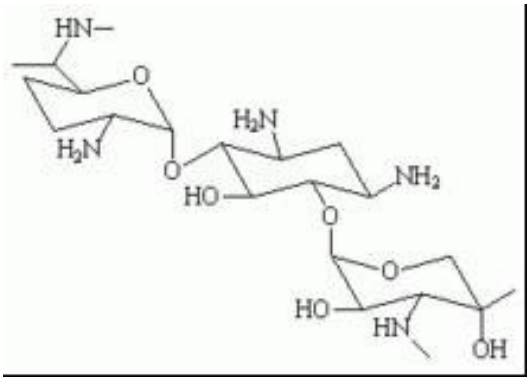
Gentamicin is one of the few heat-stable antibiotics that remain active even after autoclaving, which makes it particularly useful in the preparation of certain microbiological growth media.

Side effects

All aminoglycosides are toxic to the ear, but they vary greatly in their relative effects on hearing versus balance. Gentamicin is a vestibulotoxin, and can occasionally cause permanent loss of equilibrioception, caused by damage to the vestibular apparatus of the inner ear. Gentamicin almost never impairs hearing. In most instances, the affected individual has undergone treatment for 2 weeks or more. A small number of affected individuals have a normally harmless mutation in their mitochondrial RNA, which allows the gentamicin to affect their cells. The cells of the ear are particularly sensitive to this. Gentamicin is sometimes used intentionally for this purpose in severe Ménière's disease, to disable the vestibular apparatus.

Gentamicin can also be highly nephrotoxic, particularly if multiple doses accumulate over a course of treatment. For this reason gentamicin is usually dosed by body weight. Various formulae exist for calculating gentamicin dosage. Also serum levels of gentamicin are monitored during treatment.

Figure 7 Gentamicin



Literature reviews on Gentamicin dosage

British National Formulary (BNF) 41 March 2001, 270

Dose: by intramuscular or by slow intravenous injection over at least 3 minutes or by intravenous infusion, 2-5 mg/kg daily (in divided doses every 8 hours). By intrathecal injection, seek specialist advice, 1 mg daily (increase if necessary to 5 mg daily).The dose of gentamicin for most infections is up to 5 mg/kg daily given in divided doses every 8 hours; whenever possible treatment should not exceed 7 days. Higher doses are occasionally indicated for serious infections. Loading and maintenance doses may be calculated on the basis of the patient's weight and renal function.

International Programme on Chemical Safety INCHEM

In patients with normal renal function: 2 to 5 mg/kg daily in divided doses eight-hourly.

In patients with impaired renal function: serum concentrations of gentamicin must be measured during therapy, and dosage adjusted to give peak concentrations below 10 mg/l and trough concentrations below 2 mg/l.

The dose interval should be increased to 12 hours when creatinine clearance (CCr) is 30 to 70 ml/minute; to 24 hours when CCr is 10 to 30 ml/minute; to 48 hours when CCr is 5 to 10 ml/minute; and three to four days after dialysis at 5 ml/minute. In a usual ten-day course, the peak and trough

gentamicin level in serum must be measured at least once. If no facilities for measurement are available it is better not to continue treatment beyond 72 hours. Intrathecal administration: 1 to 5 mg/day in divided doses every eight hours, with concurrent intramuscular administration of 2 to 4 mg/kg/day.

Gentamicin containing Surgical Bone Cement, *In vitro* Elution Characteristics of Palacos and Palamed

The release of gentamicin from the two stated bone cements was measured. Gentamicin levels and peak concentration are consistently higher with use of Palamed. The peak gentamicin level at 10 minutes was 93.9 ± 16.2. The mean gentamicin concentration from 100 minutes was 5.73 ± 0.38. The percentage release was 27.1.

Release of Gentamicin from Acrylic Bone Cement

Clinical Pharmacology, 17 (4): 291-297, 1989

The pharmacokinetics of gentamicin were studied after total hip joint arthropasties performed with "Palacos R plus gentamicin" in 10 patients. The mean percentage of total released was 5.78% of the quantity implanted. The calculated peak blood concentration was 0.12 mg/L. Use acrylic bone cement containing 500 mg in 60g of mixed polymer and monomer

Release of Gentamicin from Acrylic Bone Cement

The Journal of Bone and Joint Surgery, Volume 70-A, No. 10 1988

In vitro elution studies were performed on injection moulded rods of methylmethacrylate that had been loaded with two different amounts of gentamicin. The first group of rods contained 0.5 g of gentamicin for each packet and the second 1.5 g for each packet.

The *In vitro* Elution Characteristics of Antibiotic-Loaded CMW and Palacos-R Bone Cements

The Journal of Arthroplasty, vol 14, no 2, 1999

An *in vitro* study was carried out comparing the elution characteristics of Palacos-R and CMW acrylic cements. Three groups of six antibiotic-loaded cement disks were prepared, incorporating 1 g vancomycin and 2.4 g tobramycin per 40g packet of cement. The in vitro elution characteristics of Palacos-R are superior to CMW.

Gentamicin sulphate release from a modified commercial acrylic surgical radiopaque bone cement. I. Influence of the gentamicin concentration on the release process mechanism.

Chem. Pharm Bull (Tokyo), 2002, 50, 1201-1208

The results obtained in the experiments show that the amount of gentamicin sulphate incorporated to the bone cement has a dramatic effect on the release mechanism of the drug. Therefore the relative

rate can be modified by the amount of drug incorporated.

The *in vitro* elution of gentamicin sulfate from a commercially available gentamicin-loaded acrylic bone cement, VersaBond AB.

Journal of Biomedical Material Research B Applied Biomaterials, 2004, 15, 77-83.

The *in vitro* rate of elution of getamicin sulphate from dynamically loaded specimens of VersaBond acrylic bone cement was found to be about four times that from statically loaded ones. Bulk porosity was of 8.68 ± 0.15%.

The treatment of osteomyelitis with gentamicin-reconstituted bone xenograft-composite

The Journal of Bone & Joint Surgery, 1063-1068, 2001

Used reconstituted bone xenograft-composite to treat chronic oeseomyelitis. The reconstituted bone xenograft was impregnated in 2 mg of gentamicin solution for 24 hours, then freeze dried. The pharmacokinetics of the composite *in vivo* showed that therapeutic concentrations of antibiotic remained at the site of implantation for ten days, which was sufficient to provide antimicrobial activity.

Formation Of *Propionibacterium Acnes* Biofilms On Orthopaedic Biomaterials And Their Susceptibility To Antimicrobials

Biomaterials 24 (2003) 3211-3227

In this study the bacterial growth on hip implants was measured. Gentamicin was found to be effective against biofilm-grown *Propionibacterium acnes*.

Biofilm formation by bacteria isolated from retrieved failed prosthetic hip implants in an in vitro model of hip arthroplasty antibiotic prophylaxis

Journal of Orthopaedic Research, 25:2-10, 2007

Gentamicin-loaded bone cement completely prevented colonization and biofilm formation by the *Propionibacterium acnes* isolates at 24, 48 and 72 hours. The total release time was 72 hours. Gentamicin release from the bone cement was rapid during the first 6 hours and continued at a much lower rate thereafter.

Use gentamicin sulphate, expressed as relative to the surface area of the cement section

8 h	14 µg/cm^2
24 h	16 µg/cm^2
46 h	17 µg/cm^2
72 h	18 µg/cm^2

***In Vitro* Testing of Antimicrobial Activity of Bone Cement**

Antimicrobial Agents and Chemotherapy, 2004, 4084-4088

Bone cement samples devoid of antibacterial agents, loaded with 2% gentamicin or with different concentrations of high-porosity silver.

***In Vivo* and *In Vitro* Studies of Antibiotic Release from and Bacterial Growth Inhibition by Antibiotic-Impregnated Polymethylmethacrylate Hip Spacers**

Antimicrobial Agents and Chemotherapy, 2006, 332-335

The antimicrobial properties and the elution characteristics of gentamicin-loaded hip spacers were studied. Gentamicin – release ranging from 1.52-2.84 µg for period of 14 days.

Comparable Efficacies of the Antimicrobial Peptide Human Lactoferrin 1-11 and Gentamicin in a Chronic Methicillin-Resistant *Staphylococcus aureus* Osteomyelitis Model

Antimicrobial Agents and Chemotherapy, 2005, 2438-2444

In this study 50 mg/g of gentamicin was incorporated into a calcium phosphate bone cement and injected into the debrided tibial cavity, creating a local drug delivery system. The length of study was 21 days to combat MRSA.

In vitro gentamicin release from commercially available calcium-phosphate bone substitutes influence of carrier type on duration of the release profile

BMC Muscularoskeletal Disorders 2006, 7:18

Polymethyl-methacrylate (PMMA) beads releasing antibiotics used to treat oesteomyletis. Maximum length in time for release of gentamicin was 17 days for *Chronos* calcium-phosphate bone cement. The relative release of the cements was 36-85%. Gentamicin sulphate loaded cements were produced by mixing cement powder and liquid containing 30 mg gentamicin sulphate per gram powder.

The release of gentamicin from polymethylmethacrylate beads

The Journal of Bone & Joint Surgery, 270-275, 1978

The beads used were 7 mm in diameter and weighed 0.2 g each contained 4.5 mg of gentamicin base and 20 mg of zirconium dioxide as a radiographic contrast medium. In total 30 beads were used for the study. For the *in vitro* studies 400 to 600 µg of base per day are released from one bead, a rate which falls to 10 µg by day 80.

Aminoglycosides: A practical review

Clinical Pharmacology, Vol 58, No 8, 1998

Gentamicin is the aminoglycoside used most often because of its low cost and reliable activity against gram-negative aerobes.

Cost of Gentamicin Used for Treatment of Gram-Negative Infections

Drug	Intravenous regimen			Cost*
Gentamicin	400	mg	daily	$5.00 (per 80 mg)

*--Estimated cost to the pharmacist based on average wholesale prices (rounded to the nearest half dollar) in Red book. Montvale, N.J.: Medical Economics Data, 1998. Cost to the patient will be greater, depending on prescription filling fee. Costs listed in this table are for brand names and for one day's therapy.

Single Daily Dosing of Gentamicin in Adults with Dosing Interval Adjusted for Creatinine Clearance*

Drug	Dosage (mg per kg)†	CrCl: >60 mL per minute	CrCl: 40 to 59 mL per minute	CrCl: 20 to 39 mL per minute	CrCl: <20 mL per minute
Gentamicin	5 to 7	Every 24 hours	Every 36 hours	Every 48 hours	NR

CrCl=creatinine clearance; NR=not recommended, use traditional dosing.

*--Estimate creatinine clearance as follows:

Men: CrCl=140 age/serum creatinine

Women: CrCl=140 age/serum creatinine 3 0.85

ALTERNATE FORMULA

Men: [weight (kg) 3 (140 age)] / 72 3 serum creatinine (mg per dL)

Women: 0.85 3 calculation for men

†--Adjust dosage for obese patients (>30% ideal body weight [IBW]) as follows:

Women: IBW=45 kg + 2.3 kg for every inch above 5 feet

Men: IBW=50 kg + 2.3 kg for every inch above 5 feet

Dosing weight=IBW + (total body weight IBW) 0.4

ALTERNATE FORMULA

Women: 100 lb + 5 lb for every inch above 5 feet

Men: 110 lb + 5 lb for every inch above 5 feet

NOTE: Single daily dosing is currently not recommended for paediatric patients, burn patients, or patients with cystic fibrosis, enterococcal infections or bacterial endocarditis.

Values for Monitoring Gentamicin Serum Concentration Levels When Using the Single Daily Dosing Method of Administration*

Drug	Serum concentration level for dosing every 24 hours	Serum concentration level for dosing every 36 hours	Serum concentration level for dosing every 48 hours	Traditional method preferred (µg per mL)	Expected trough, before next dose (µg

	(µg per mL)	(µg per mL)	(µg per mL)		per mL)
Gentamicin†	<3	3 to 5	5 to 7	>7	<0.5 to 1.0

*--Check the patient's aminoglycoside serum concentration level (µg per mL) 12 hours after the start of the 60-minute transfusion. Adjust the dosing interval according to the results of the serum concentration level. Example: if level is 4 for gentamicin, change dosing to every 36 hours; if level is 2, keep dosing at every 24 hours; if level is 8, switch to traditional dosing method.
†--A once-daily nomogram may also be used if serum concentration is measured at time other than 12 hours.

Traditional Multiple Daily Dosing of Aminoglycosides in Adults*

Drug: route	Loading dose† (mg per kg)	Maintenance dose† (mg per kg)	Age: <60 and CrCl: >90 mL per minute	Age: >60 or CrCl: 50 to 90 mL per minute	CrCl: 10 to 50 mL per minute
Gentamicin: IV/IM	2 to 3	1.7	Every 8 hours	Every 12 hours	Every 24 to 48 hours

CrCl=creatinine clearance; IV=intravenously; IM=intramuscularly.
*--Dosing interval based on estimated creatinine clearance (mg per mL) and/or age (years). Estimation of creatinine clearance as follows:
Men: CrCl=140 age/serum creatinine
Women: CrCl=140 age/serum creatinine 3 0.85
ALTERNATE FORMULA:
Men: [weight (kg) 3 (140 age)] / 72 3 serum creatinine (mg per dL)
Women: 0.85 3 calculation for men
†--Adjust dosage for obese patients (>30% ideal body weight [IBW]) as follows:
Women: IBW=45 kg + 2.3 kg for every inch above 5 feet
Men: IBW=50 kg + 2.3 kg for every inch above 5 feet
Dosing weight=IBW + (total body weight IBW) 0.4
ALTERNATE FORMULA:
Women: 100 lb + 5 lb for every inch above 5 feet
Men: 110 lb + 5 lb for every inch above 5 feet

Gentamicin release from old cement during revision hip arthroplasty

The Journal of Bone & Joint Surgery, 607-610, 1998

Sensitive strains of *Staphylococus* are inhibited by concentrations of gentamicin of less then 1 mg/l. Gentamicin is rapidly bactericidal for these organisms at concentrations above the minimum inhibitory concentration in non-resistant strains. Gentamicin may be present in the tissues in very high concentrations which far exceed those required to kill the organisms that are responsible for most infections. In one of the patients the gentamicin concentration in the joint fluid sampled before the cement had been cracked was very high (92.3 mg/l).

Release of gentamicin and vancomycin from temporary human hip spacers in two-stage revision of infected arthroplasty

The Journal of Antimicrobial Chemotherapy, 2004, 53, 329-334

After 12-24 weeks in the hip, the removed spacers still released appreciable amounts (850-1800 µg) of gentamicin, representing (0.05-0.09% of the initial total amount and in the range 4.7-10 µg/cm^2. In the first 24 hours the elution of gentamicin was 1285.4 ± 563.3 µg (mean ± standard deviation). The total release of gentamicin was 23.0 ± 11.1 µg/cm^2, after several weeks. The average total release was 1308.3 ± 337 µg. The average release per area was 7.3 ± 1.9 µg/cm^2.

Quantitation of slow drug release from an implantable and degradable gentamicin conjugate by *in vivo* magnetic resonance imaging

Antimicrobial Agents and Chemotherapy, 1995, 839-845

Gentamicin-hydrogel samples containing 650 µg of gentamicin each incubated in rat whole plasma showed liberation of gentamicin in a time-dependent fashion. At 120 hours incubation, 327 ± 18.9 µg (50.7% of total gentamicin) had been released.

Bisphosphonates

Bisphosphonates is a class of drugs that inhibits the resorption of bone. Its uses include the prevention and treatment of osteoporosis, osteitis deformans ('Paget's disease of bone'), bone metastasis (with or without hypercalcemia), multiple myeloma and other conditions that feature bone fragility.

Chemistry and classes:

All bisphosphonate drugs share a common P-C-P 'backbone': The two PO_3 (phosphate) groups covalently linked to carbon determine both the name 'bis*phospho*nate' and the function of the drugs. The long *side chain* (R_2 in Figure 8) determines the chemical properties, the mode of action and the strength of bisphosphonate drugs. The short side chain (R_1), often called the 'hook,' mainly influences chemical properties and pharmacokinetics.

Pharmacokinetics:

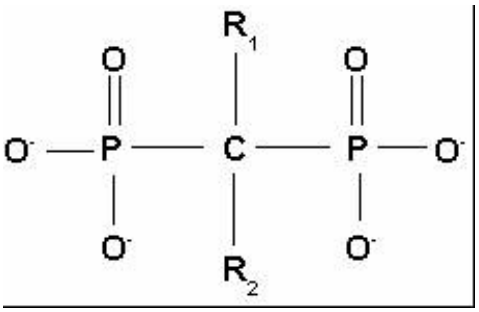
Of the bisphosphonate that is resorbed (from oral preparation) or infused (for intravenous drugs), about 50% is excreted unchanged by the kidney. The remainder has a very high affinity for bone tissue, and is rapidly absorbed onto the bone surface.

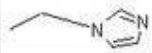
Mechanism of action:

Bisphosphonates, when attached to bone tissue, are 'ingested' by osteoclasts, the bone cell that breaks down bone tissue.

There are two classes of *bisphosphonate*: the *N*-containing and non-*N*-containing bisphosphonates. The two types of bisphosphonates work differer

Figure 8 General Formula for Bisphosph



Agent	R_1 side chain	R_2 side chain
Etidronate	-OH	$-CH_3$
Clodronate	-Cl	-Cl
Tiludronate	-H	-S-  -Cl
Pamidronate	-OH	$-CH_2-CH_2-NH_2$
Neridronate	-OH	$-(CH_2)_5-NH_2$
Olpadronate	-OH	$-(CH_2)_2N(CH_3)_2$
Alendronate	-OH	$-(CH_2)_3-NH_2$
Ibandronate	-OH	$-CH_2-CH_2N$ (CH_3, $(CH_2)_4-CH_3$)
Risedronate	-OH	
Zoledronate	-OH	

Non-nitrogenous:

Non-*N*-containing bisphosphonates:

- Etidronate (Didronel®) - 1 (potency relative to that of etidronate)
- Clodronate (Bonefos®, Loron®) - 10
- Tiludronate (Skelid®) - 10

The non-nitrogenous bisphosphonates are metabolised in the cell to compounds that compete with adenosine triphosphate (ATP) in the cellular energy metabolism. The osteoclast initiates apoptosis and dies, leading to an overall decrease in the breakdown of bone.

Nitrogenous:

N-containing bisphosphonates:

- Pamidronate (APD, Aredia®) - 100
- Neridronate - 100
- Olpadronate - 500
- Alendronate (Fosamax®) - 500
- Ibandronate (Bondronat®) - 1000
- Risedronate (Actonel®) - 2000
- Zoledronate (Zometa®) - 10000

Nitrogenous bisphosphonates act on bone metabolism by binding and blocking the enzyme farnesyl diphosphate synthase (FPPS) in the HMG-CoA reductase pathway (also known as the mevalonate pathway).

Uses:

Bisphosphonates are used clinically for the treatment of osteoporosis, osteitis deformans (Paget's disease of the bone), bone metastasis (with or without hypercalcemia), multiple myeloma and other conditions that feature bone fragility.

In osteoporosis and Paget's, alendronate and risedronate are the most popular first-line drugs. If these are ineffective or the patient develops digestive tract problems, intravenous pamidronate may be used. Alternatively, strontium ranelate or teriparatide are used for refractory disease, and the SERM raloxifene is occasionally administered in postmenopausal women instead of bisphosphonates.

High-potency intravenous bisphosphonates have shown to modify progression of skeletal metastasis in several forms of cancer, especially breast cancer. More recently, bisphosphonates have been used to reduce fracture rates in children with osteogenesis imperfecta.

Side-effects:

- Oral bisphosphonates can give stomach upset and inflammation and erosions of the oesophagus, which is the main problem of oral *N*-containing preparations. This can be prevented by remaining seated upright for 30 to 60 minutes after taking the medication.
- Intravenous bisphosphonates can give fever and flu-like symptoms after the first infusion, which is thought to occur because of their potential to activate human γδ T cells. Notably, these symptoms do not recur with subsequent infusions.
- There is a slightly increased risk for electrolyte disturbances, but not enough to warrant regular monitoring.
- In chronic renal failure, the drugs are excreted much slower, and dose adjustment is required.
- Bisphosphonates have been associated with osteonecrosis of the jaw; with the mandible twice as frequently affected as the maxilla and most cases occurring following high-dose intravenous administration used for some cancer patients. Some 60% of cases are preceded by a dental surgical procedure and it has been suggested that bisphosphonate treatment should be postponed until after any dental work to eliminate potential sites of infection.
- A number of cases of severe bone, joint or musculoskeletal pain have been reported, prompting labelling changes.

Literature reviews on Bisphosphonate dosage and application time

1. B. Peter, D. P. Pioletti, S. Laib, B. Bujoli, P. Pilet, P. Janvier, J. Guicheux, P.-Y. Zambelli, J.-M. Bouler, O. Gauthier.

Calcium phosphate drug delivery system: influence of local zolendronate release on bone implant osteointegration.
Bone 36 (2005), 52-60.

Zolendronate was grafted to HA coating of titanium implants in various concentrations (0,2µg/implant, 2,1µg/implant, 8,5µg/implant and 16µg/implant). Rats.

Results 3weeks: SEM, bone density

20µm around the implant

0,2 and 2,1µg/l result in the highest bone densities decreasing with distance
8,5µg/l shows a lower but steadier density
16µg/l and control (without Zolendronate) show the lowest density. 16µg/l with increases bone density with the distance, control-decreases with the distance

40-80µm

2,1 µg/l shows the highest bone density

80-200µm
2,1 to 16µg/l converges to a common highest density

Results 3weeks: pullout

The maximal pullout force increases up to 2,1µg/l. By further increasing the Zolendronate content of the coating, the pullout force decreases and reaches levels lower when no zolendronate is present.

2. B. Peter, O. Gauthier, S. Laib, B Bujoli, J. Guicheux, P. Janvier, G. H. van Lenthe, R. Muller, P. Zambelli, J. Bouler, D. Pioletti.

Local delivery of bisphosphonate from coated orthopaedic implants increases implants mechanical stability in osteoporotic rats. 2005, jbm.a.30456

Zolendronate was grafted to HA coating of titanium implants in various concentrations (0,2µg/implant, 2,1µg/implant, 8,5µg/implant and 16µg/implant). Ovariectomized rats.

Results 3weeks: SEM

The densities 20µm around the implant loaded with any Zolendronate content was higher than the bone volume fraction around the implant without Z. Three lowest Z. contents (0, 0.2, 2,1µg/implant) decreased with the distance. Two highest Z. doses increased the bone volume fraction with the distance.

Results 3weeks: histomorphometry

The implant coated with 0,2µg/implant significantly influenced all the histomorphometric parameters.

Results 3weeks: µ-CT

The implants containing the two highest Z. contents generate bone densities which are significantly higher than the bone densities generated around the implants containing 0 and 0,2µg of Z. The coating loaded with 2,1µg of Z. generates a bone significantly denser than the bone around implants containing 0,2µg.

Results 3weeks: pullout

Existence of a window of Zolendronate content (0,2µg/l to 8,5µg/l) in which the mechanical fixation of the implant increased.

3. Jörgen Åstrand, Per Aspenberg.
Topical, single dose bisphosphonate treatment reduced bone resorption in a rat model for prosthetic loosening. J Orthopaedic Res 22 (2004), 244-249.

20µl of 1mg/ml alendronate solution was applied as a single dose in the bone adjacent to the titanium implant plate. Bone resorbtion model.

Results: histology

Conclusion: rats treated with alendronate had soft tissue areas at the interface reduced by half.

4. B. Skoglund, J. Holmertz, P. Aspenberg.
Systemic and Local ibandronate enhance screw fixation. J Orthopaedic Res, 2004, 22, 1108-1113.

Local application: 0,1ml of ibandronate (0,1mg) (or saline) was injected as a single dose into the drilled hole before immediate stainless steel screws insertion in rats.

Results 14 days: pullout:

Local applied Ibandronate resulted in 15% larger force at failure than controls. Systemic ibandronate increased the pull-out by 30%.

Results 14 days: torque-moment:

Local Ibandronate resulted in a 60% larger torque-moment than controls.

5. P. Tengvall, B.Skoglund, A. Askendal, P. Aspenberg.
Surface immobilized bisphosphonate improves stainless-steel screw fixation in rats.
Biomaterials 25 (2004) 2133-2138.

Pamidronate /1mg/ml in distilled water) was immobilized onto fibrinogen on Ti and ibandronate (50µg/ml in distilled water) was adsorbed on top of this. 216 ng/cm2 as measured by ellipsometry.

Results 2weeks: pullout:
28% higher pullout force and 90% increased pullout energy for the bisphosphonate coated screws.

6. T. Jakobsen, S. Kold, J. Bechtold, B. Elmengaard and K. Soballe.
Effect of Topical Alendronate Treatment on fixation of implant inserted with bone compaction.
Clinical Orthopaedics and related research, 444, 229-234.

Porous-coated titanium implants were inserted with bone compaction into the knees of dogs. Topical BP (alendronate 15 cc) treatment was applied before bone compaction.

Results 4 weeks: histomorphometry and biomechanical studies.
An increase in total bone-to-implant contact and total bone density was found. No change in biomechanical fixation (push out test) was found.

7. S. Meraw, C. Reeve, P. Wollan.
Use of Alendronate in peri-implant defect regeneration. J. Periodontol, February 1999, 151-158.

HA- coated implants (were soaked in 0,1mmol sterile solution of alendronate for one week and titanium machine-polished implants (with 2,8µg of alendronate) were inserted in dogs.

Results 28 days: fluorescence and histomorphometri:
Increased bone formation rate in both groups. Increased Bone-to implant contact in the TMP model and decrease in HA model.

Other references:

8. A. H. A. Kurth, C. Eberhardt, S. Muller, M. Steinacker, M. Schwarz and F. Bauss.
The bisphosphonate ibandronate improves implant integration in osteopenic ovariectomized rats. Bone 37 (2005) 204-210.

Daily subcutaneous injections ibandronate at a dose of 1µg/kg or 25 µg/kg.

Sterilisation Methods

Here listed are potential sterilisation methods to be used in this project. Sterilisation and packaging in

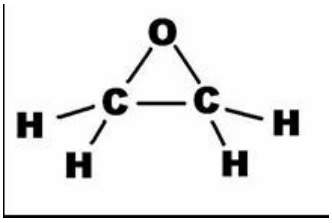
the project will be performed by Hunt, form their expertise in the area will able to select the right type of sterilisation to use.

Chemical sterilisation

Chemicals are used for sterilization. Although heating provides the most effective way to rid an object of all transmissible agents, it is not always appropriate, because it destroys objects such as most fibre optics, most electronics, and some plastics.

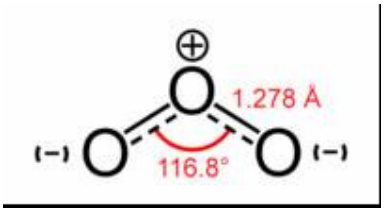
Ethylene oxide (EtO) gas is commonly used to sterilise objects that cannot survive temperatures greater than 60℃ such as plastics, optics and electrics. Ethylene oxide treatment is generally carried out between 30℃ and 60℃ with relative humidity above 30% and a gas concentration between 200 mg/l and 800 mg/l for at least 3 hours. Ethylene oxide penetrates very well, moving through paper, cloth, and some plastic films and is highly effective. Ethylene oxide however is highly flammable, and requires a longer time to sterilise than any heat treatment. The process also requires time for aeration post sterilization to remove toxic residues. Ethylene oxide is widely used and sterilises around 50% of all disposable medical devices.

Figure 9 Ethylene oxide



A rapid biological indicator is available for use in EtO sterilisers. This indicator contains *Bacillus subtilis*, which is a very resistant organism. If sterilization fails, incubation at 37℃ will cause a fluorescent change within four hours, which is read by an auto-reader. After 96 hours, a visible colour change will occur. The fluorescence is emitted when a particular (EtO resistant) enzyme is present, which means that spores are still active. The colour change is brought on by a pH shift due to bacterial metabolism. The test is suitable for most types of ethylene oxide cycles. The rapid results mean that if a cycle was found to be ineffective, the objects treated can be quarantined and physicians quickly advised of possible contamination.

Figure 10 Ozone

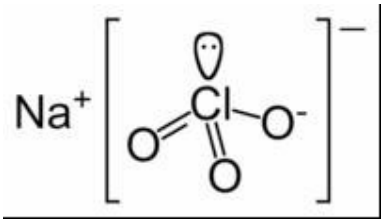


Ozone is used in industrial settings to sterilise water and air, as well as a disinfectant for surfaces. It has the benefit of being able to oxidize most organic matter. On the other hand, it is a toxic and unstable gas that must be produced on-site, so it is not practical to use in many settings.

Bleach is another accepted liquid sterilizing agent. Household bleach, also used in hospitals and

biological research laboratories, consists of 5.25% sodium hypochlorite. At this concentration it is most stable for storage, but not most active. According to the Beth Israel Deaconess Medical Centre Bio-safety Manual (2004 edition), in most cases, it should be diluted to 1/10 of its storage concentration immediately before use; however, it should be diluted only to 1/5 of the storage concentration to kill *Mycobacterium tuberculosis*. This dilution factor must take into account the volume of any liquid waste that it is being used to sterilise.

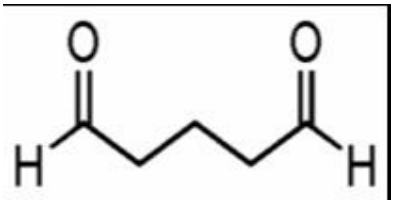
Figure 11 Sodium hypochlorite



Bleach will kill many organisms immediately, but should be allowed to incubate for 20 minutes for full sterilization. Bleach will kill many spores, but is ineffective against certain extremely resistant spores. It is highly corrosive, even causing rust of stainless steel surgical implements.

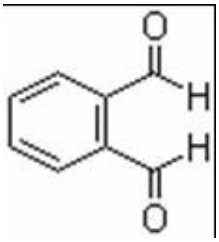
Glutaraldehyde and formaldehyde solutions (also used as fixatives) are additional accepted liquid sterilizing agents, provided that the immersion time is long enough – it can take up to 12 hours for glutaraldehyde to kill all spores, and even longer for formaldehyde. (This assumes that a liquid not containing large solid particles is being sterilised. Sterilization of large blocks of tissue can take much longer, due to the time required for the fixative to penetrate.)

Figure 12 Glutaraldehyde



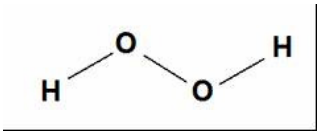
Glutaraldehyde and formaldehyde are volatile, and toxic by both skin contact and inhalation. Glutaraldehyde has quite a short shelf life (<2 weeks), and is expensive. Formaldehyde is less expensive and has a much longer shelf life if some methanol is added to inhibit polymerization to paraformaldehyde, but is much more volatile. Formaldehyde is also used as a gaseous sterilizing agent; in this case, it is prepared on-site by depolymerisation of solid paraformaldehyde.

Figure 13 Ortho-phthalaldehyde



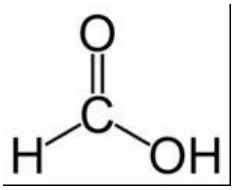
Ortho-phthalaldehyde (OPA) is a sterilizing chemical which received Food and Drug Administration (FDA) clearance in late 1999. Typically used in a 0.55% solution, OPA shows better myco-bactericidal activity than glutaraldehyde. It also is effective against glutaraldehyde-resistant spores. OPA has superior stability, is less volatile, and does not irritate skin or eyes, and it acts more quickly than glutaraldehyde. On the other hand, it is more expensive, and will stain proteins (including skin) grey in colour.

Figure 14 Hydrogen peroxide



Another chemical sterilizing agent is hydrogen peroxide. It is relatively non-toxic once diluted to low concentrations (although a dangerous oxidizer at high concentrations), and leaves no residue. The Sterrad 50 and other Sterrad sterilization chambers use hydrogen peroxide vapour to sterilise heat-sensitive equipment such as rigid endoscopes. The Sterrad 50 sterilises in 45 minutes and also penetrates some lumen devices. The most recent Sterrad model, Sterrad NX, can sterilise most hospital loads in as little as 20 minutes and has greatly expanded lumen claims compared to earlier models. The Sterrad has limitations with processing certain materials such as paper/linens and long thin lumens. Paper products cannot be sterilised in the Sterrad system because of a process called cellulostics, in which the hydrogen peroxide would be completely absorbed by the paper product.

Figure 15 Formic acid



Endoclens is another device used to sterilise endoscopes. It mixes two chemicals (hydrogen peroxide and formic acid) together to make its antiseptic as needed. The machine has two independent asynchronous bays, and cleans (in warm detergent with pulsed air), sterilises and dries the endoscopes automatically. All air and water inlets are filtered, and the machine handles temperature, timing and chemical concentration. The total time for the whole process is 30 minutes, and a hard-copy report of the cycle is printed (as well as being stored electronically). Studies with synthetic soil containing bacterial spores showed this machine achieved sterilization effectively.

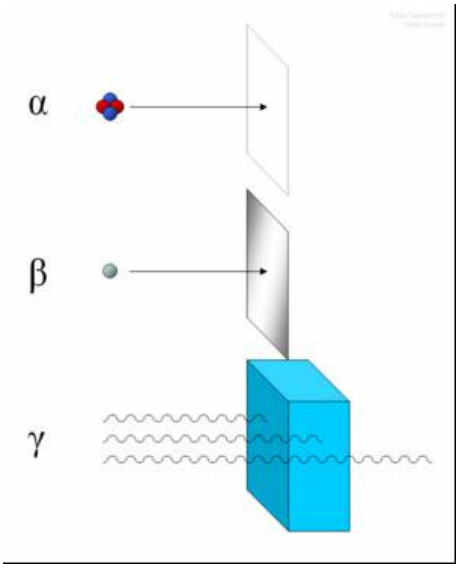
The Dry Sterilization Process, DSP, is a process originally designed for the sterilization of plastic bottles in the beverage industry. It uses hydrogen peroxide with a concentration of 30-35% and runs under vacuum conditions. Using the common reference germs for hydrogen peroxide sterilization processes, endospores of different strains of *bacillus subtilis* and *bacillus stearothermophilus*, the Dry Sterilization Process achieves a germ reduction of 10^6 to 10^8. The complete cycle time of the process is 6 seconds. The surface temperature of the sterilised items is only slightly increased during the process by 10 to 15°C. Particularly due to the high germ reduction and the slight temperature increase the Dry Sterilization Process is also useful for medical and pharmaceutical applications.

Radiation sterilisation

Methods exist to sterilise using radiation such as X-rays, gamma rays, or subatomic particles.

Figure 16 Alpha radiation consists of helium nuclei and is readily stopped by a sheet of paper. Beta radiation, consisting of electrons, is halted by an aluminium plate.

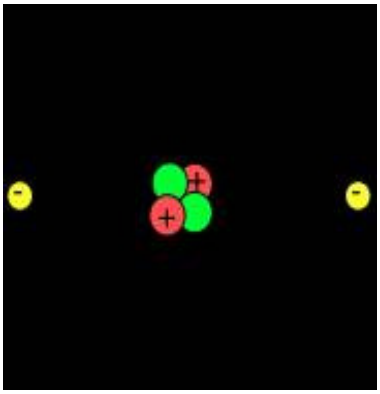
Gamma radiation is eventually absorbed as it penetrates a dense material.



Gamma rays are very penetrating, but as a result require bulky shielding for the safety of the operators of the gamma irradiation facility; they also require storage of a radioisotope, which continuously emits gamma rays (it cannot be turned off, and therefore always presents a hazard in the area of the facility). X-rays are less penetrating and tend to require longer exposure times, but require less shielding, and are generated by an X-ray machine that can be turned off for servicing.

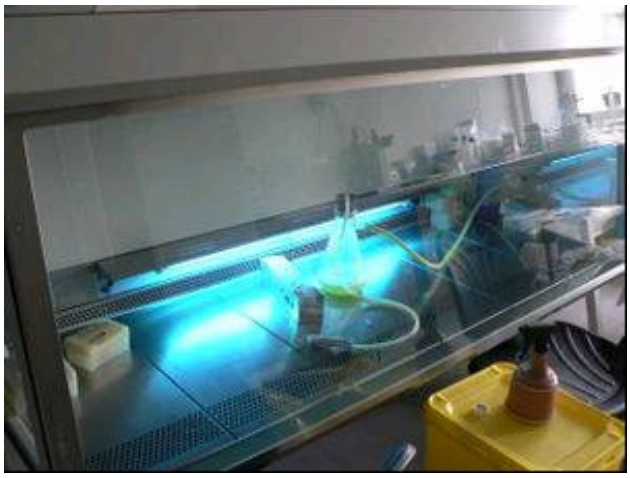
Subatomic particles may be more or less penetrating, and may be generated by a radioisotope or a device, depending upon the type of particle. Irradiation with X-rays or gamma rays does not make materials radioactive. Irradiation with particles may make materials radioactive, depending upon the type of particles and their energy, and the type of target material: neutrons and very high-energy particles can make materials radioactive, but have good penetration, whereas lower energy particles (other than neutrons) cannot make materials radioactive, but have poorer penetration.

Figure 17 A schematic representation of the subatomic helium atom; showing two protons (red), two neutrons (green) and two electrons (yellow).



Ultraviolet light (UV, from a germicidal lamp) can also be used for irradiation, but only on surfaces and some transparent objects (note that many objects that are transparent to visible light actually absorb UV). It is routinely used to sterilise the interiors of biological safety cabinets between uses, but is ineffective in shaded areas, including areas under dirt (which may become polymerised after prolonged irradiation, so that it is very difficult to remove). It also damages many plastics, as can be seen if one forgets a polystyrene foam object in the cabinet with the germicidal lamp turned on overnight.

Figure 18 A low pressure mercury vapour discharge tube floods the inside of a hood with shortwave UV light when not in use, sterilizing microbiological contaminants from irradiated surfaces.



Results and Discussion

The drugs to be used in this study would need to able to help treat the following bone disorder diseases; osteoporosis, osteitis deformans, bone metastasis and multiple myeloma. The background for all of these disorders has been researched. The analysis shows that an antibiotic drug would be suitable for this application to fight harmful infection. A bone promoting drug would also help as this would encourage the re-growth of damaged bone tissue.

The drug candidates selected to help treat these disorders are; gentamicin (antibiotic) and a bisphosphonate.

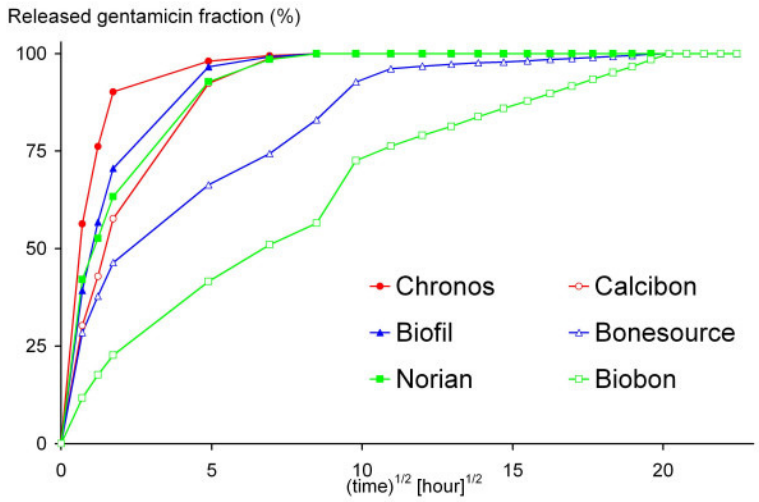
As discussed earlier gentamicin is one of the most common antibiotics used during various types of bone surgery. So was therefore also aptly selected in our research. It is readily available from drug manufactures to be used in our research. Gentamicin is often used in bone cement (Palacos® by Biomet and SMARTSET GHV by Johnson & Johnson). The thorough literature reviews show it availability and use as a drug during surgery with dosage information and release information. The results of the research show varying degrees of gentamicin being used and dosage depends on the weight and metabolism of the patient along with other important factors.

It was concluded that the recommended gentamicin dosage fro this research should be 5 mg/kg a day over an 8 hour period, anything greater than this could be harmful in humans. This dosage information and evidence was taken from the British National Formulary and the International Programme on Chemical Safety. Higher doses are occasionally indicated for serious infections.

Loading and maintenance doses may be calculated on the basis of the patient's weight and renal function.

A literature review (Stallmann *et al.* BMC Musculoskeletal Disorders 2006 7:18) on the gentamicin release rate shows different products with varying release characteristics (Graph 1). They recommended that the Norian and Calicibon products containing product had the most promising gentamicin release ability. Both released 100% of the gentamicin content in about the 8 hours timescale, which is approved by the BNF and IPCS. This release rate should be emulated for our research, so the aim would be to release the encapsulated gentamicin in a similar timescale. The graph shows a flash release of gentamicn which gradually slows down over time. After 8 hours the curve is horizontal illustrating a complete release.

Graph 1: Release profile of gentamicin required



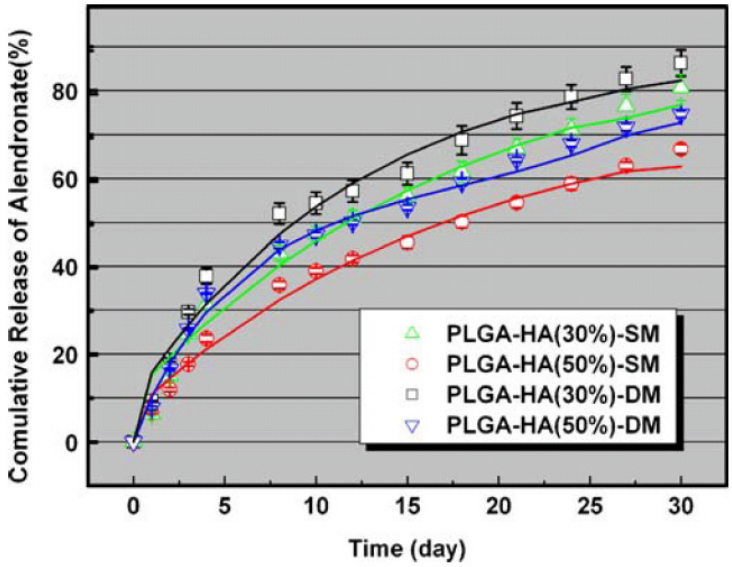
It was concluded by Gothenburg University that the recommended bisphosphonate dosage for this research should be 2.1µg-8.5µg per implant, anything greater than this could be harmful in humans and produce negative effects.

This dosage information and evidence was taken from their thorough literature searches and experience in the bone osteoporosis research area. They confirmed the most studied

bisphosphonates were Zolendronate, Pamindronate, Ibandronate and Alendronate and concluded that one of these should be used for this study. Alendronate was selected as there are many references to its use in the literature and fewer cases of side-effects and toxicity.

Research in a paper in Pharmaceutical Research (Pharm Res. Feb;26(2):422-30), shows the release of alendronate from PLGA microshperes for bone repair. It was envisaged that we should also aim to have a similar release rate (Graph 2). Release rate for the alendronate should be slower and over a long period of time. It was envisaged to have a release over a 30 day period, where the majority of the drug should elute.

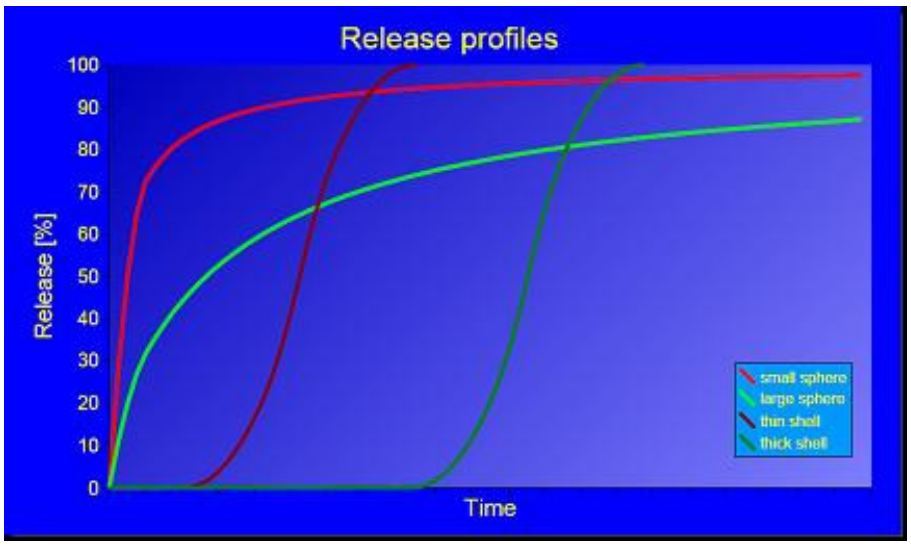
Graph 2: Cumulative release of Alendronate from PLGA / Hydroxyapaptite microspheres. Indication of the release profile required.



Brace will be using the microencapsulation process to coat the respective drug using a polymer show their release profiles (Graph 3) varying with the shell size. Microencapsulation with microspheres provides a solution to using pure active agents that have many disadvantages; there is difficulty in the application; they are unstable in air and in the digestive system; it causes a “burst” effect and the

dosage and handling is very difficult. Microspheres are solid spheres with a matrix-encapsulated active agent.

Graph 3 Release profiles of varying shell and sphere sizes.



Conclusion

Overall, a thorough search of the literature has been conducted on the use of gentamicin and bisphosphonates for bone surgery. The two drug candidates for the study are gentamicin, as the antibiotic, and alendronate a bisphosphonate, as the bone promoting drug.

The suggested dosage for gentamicin dosage for this research should be 5 mg/kg a day over an 8 hour period, anything greater than this could be harmful in humans. This dosage information and evidence was taken from the British National Formulary and the International Programme on Chemical Safety.

It was concluded by Gothenburg University that the recommended bisphosphonate dosage for this research should be 2.1µg-8.5µg per implant, anything greater than this could be harmful in humans and produce negative effects.

These drugs will be coated in to microspheres or encapsulated for the delivery of the drug. Ultimately, he coating of the microspheres will able to determine the length of time for the release of the particular drug.

Deliverable 2

Summary

The nature of this deliverable is to produce a short technical note the synthetic strategy to be developed by Brace and Pera in this research. Brace and Pera will embark upon different avenues to coat the chose drug candidates (gentamicin and alendronate) using biodegradeable and biocompatible polymers and materials.

Introduction

Pera will be using the sonication process (using ultrasonication) to encapsulate the drug material using a polymer. This will use high frequency mixing of solvent phase and water phase materials to form an emulsion. This emulsion will contain microspheres that are coating the respective drug.

Pera will encapsulate the respective drug using a mixture of PLGA and PVA – polymer encapsulation. This will be used to coat gentamcin and alendronate. Blank microspheres will also be created in the absence of a drug, for comparison purposes. They will also encapsulated the respective drug using phosphatidyl choline and cholesterol – forming a liposome. The liposome will be used to coat gentamicin and alendronate. Blank liposomes will also be created in the absence of a drug, for comparison purposes.

Table: Synthetic encapsulation strategy at Pera

Process	**Encapsulating materials**	**Drugs**
Sonication	PLGA and PVA	No drug
Sonication	PLGA and PVA	gentamcin
Sonication	PLGA and PVA	alendronate
Sonication	phosphatidyl choline and cholesterol	No drug
Sonication	phosphatidyl choline and cholesterol	gentamcin
Sonication	phosphatidyl choline and cholesterol	alendronate

Brace used methods of microencapsulation and film coating to coat the drug with a polymer. The polymers used for microencapsulation were TCP and HA, for film coating eutragit or carbopol were used. Again the two drug candidates used were gentamicin and alendronate.

For the microencapsulation using TCP and HA two different grades of powders will be used. They will be at 40 microns and finer powders for both TCP and HA at 10 microns will be used. Both types of powders will be used to microencapsulate gentamicin and alendronate. Again blank examples in the absence of a drug will be produced as a comparison.

For the film coating carbopol and eutragit polymers will be used to coat gentamicin and alendronate respectively.

Table: Synthetic encapsulation strategy at Brace

Process	**Encapsulating materials**	**Drugs**
Microencapsulation	TCP and HA, 40 microns	No drug
Microencapsulation	TCP and HA, 40 microns	gentamcin
Microencapsulation	TCP and HA, 40 microns	alendronate
Microencapsulation	TCP and HA, 10 microns	No drug
Microencapsulation	TCP and HA, 10 microns	gentamcin
Microencapsulation	TCP and HA, 10 microns	alendronate
Film coating	Carbopol	gentamcin
Film coating	Eutragit	alendronate
Film coating	Eutragit	gentamcin

Discussion

The synthetic strategies mentioned provide a wide variety of different methods to conduct the encapsulation research. Suitable polymer and drug candidates have been identified and viable processes for encapsulation are envisaged. All encapsulation materials (PLGA, PVA, TCP, HA, Carbopol and Eutragit) are biocompatible and have been used in drug delivery devices for other research. The drugs to be encapsulated will be gentamicin and alendronate. There will be 3 different methods used to encapsulate the drug; sonication, microencapsulation and film coating. All of these processes have been used in other types of research to create microparticles.

Conclusion

Overall, three different strategies have been identified to encapsulate the selected drug candidates. This work will be conducted and the data will be presented in subsequent deliverable reports. The next step would be to conduct the work and analyse the results they produce. If encapsulation is successful then work on the cytotoxicity testing and pre-clinical work may proceed.

Deliverable 3

Summary

The nature of this deliverable is to produce a short technical note describing the working basic method for synthesis of a single polymer. The synthesis of the polymer PLGA (poly(lactic-co-glycolic acid)) is described from monomer units. The synthesis of the encapsulated drug with PLGA and PVA (polyvinyl alcohol) is also mentioned using ultrasonication to create drug-loaded microspheres. The report also explains how the PLGA should degrade once on the implant and inserted in the body

Introduction

This report describes the working basic method for the synthesis of a single polymer of PLGA (poly(lactic-co-glycolic acid)). Of the polymers available, PLGA is used in the research as it is approved by the Food and Drug Administration and has previously been used in therapeutic devices because of its biocompatibility and biodegradability.

It also describes experiment that the PLGA polymer will be used for, how it coats the required drug and how it breaks down in the body once it is coated on the implant.

Experimental

PLGA is synthesised using glycolic acid and lactic acid, where the cyclic dimers are randomly ring opened in a co-polymerisation reaction. A tin 2-ethylhexanoate catalyst is used to facilitate the increase for the rate of reaction. The solvent used in the reaction is dichloromethane. During the polymerisation process the monomers of glycolic acid and lactic acid link together liked by ester bonds formed in the process. This forms the characteristic straight chain polymer.

The composition and properties of the PLGA can be altered by changing the quantities of lactide and glycolide. Therefore different forms of PLGA can be made by changing the ratios of the monomers, lactide to glycolide. PLGA 75:25 has a copolymer composition of 75% lactic acid and 25% glycolic acid. PLGA 50:50 has a copolymer composition of 50% lactic acid and 50% glycolic acid. PLGA 25:75 has a copolymer composition of 25% lactic acid and 75% glycolic acid.

The PLGA is subsequently used to encapsulate the drug using ultrasonication. Described below is a technical note for the synthesis of the encapsulated drug using existing biodegradable synthetic

polymers - PLGA.

Journal of Controlled Release, 2006
International Journal of Pharmaceutics, 314, 198-206, 2006

Gentamicin microspheres

PVA (80 mg) was dissolved in water (21 ml) and stirred
PLGA (50:50 composition) (30 mg) was dissolved in dichloromethane (3 ml)
Gentamicin sulphate (30 mg) was dissolved in the PVA solution (1 ml) and was added to the dichloromethane mixture and was sonicated (13 W output for 90 seconds) over an ice-bath.
The primary emulsion was added to the PVA solution (20 ml) and sonicated (18 W output for 90 seconds) over an ice-bath, to form a double emulsion.
The formed double emulsion was stirred for 3 hours to remove dichloromethane by evaporation.
The sample was stored in a fridge at 4ºC.

In the procedure the PLGA is sonicated with the PVA polymer in a double emulsion reaction. This enables the encapsulation of the gentamicin drug. The PLGA and PVA form a single polymer material to encase the drug successfully.

Results and Discussion

Overall, this shows that PLGA can be synthesised and used with PVA (polyvinyl alcohol) as a copolymer to encapsulate a selected drug, in this case gentamicin. It also shows that the sonication encapsulation process works well using PLGA and PVA.

The subsequent step would be to successfully encapsulated the gentamicin and then coat the microsphere particles on to the implant surface. The implant surface would be composed of hydroxyapatite. Once the implant is in the body the PLGA would degrade in the presence of aqueous bodily fluids by hydrolysis the ester linkages. The degredation time can be controlled by altering the ratio of polymers used, in the synthesis.

The time required for the degradation of PLGA is correlated with the ratio of the monomers used in its synthesis. A high ratio of glycolide units means a shorter time for its breakdown. Although the 50:50 PLGA ratio shows a faster degradation, if the polymer is capped with an ester then degradation time is increased considerably.

Once PLGA, being a biodegradable, has been adhered on the implant it is should undergo hydrolysis in the body, to return to this constituent monomer components of lactic acid and glycolic acid and producing carbon dioxide and water. Both of these monomers are by-products of different metabolic pathways, under the usual physiological conditions. As the two monomers are synonymous to breakdown products the body is able to deal with them effectively. PLGA has a very low toxicity when being used for drug delivery or biomaterial applications.

Conclusion

In conclusion, this report has explained the basic working models to be used in this research. The work shows that PLGA can be synthesised and how a copolymer is produced with PVA. The work shows the challenges and ideas needed to move forward in the research. The sonication process using ultrasonication equipment has been described in a technical procedure on what work will be performed. The encapsulated drug in the PLGA-PVA polymer once on the implant and in the body should hydrolyse the polymer to release the drug over a specific time period. From these results, the present study may help us on our way towards a better understanding of PLGA synthesis and degradation behaviour, which is very important for controlled-release of drug delivery system. This allows the research to move forward to the next consequent step which would be to coat the implants. The degradation of the PLGA polymer should allow release of the specified drug; either gentamicin or alendronate.

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Deliverable 4

Summary

The nature of this deliverable is under the category of 'prototype', so is given as a brief report showing a table of results of all the successful synthetic products developed by Brace and Pera in the research.

The table summarises the types of polymers used, the type of drug used, the size of the particles, the encapsulation method employed, the method for its adherence to the HA coating and the distribution of the polymer with the drug.

Introduction

This report shows a table summarise the range of synthetic products and methods exploiting the methods described in Task 2.2. The table shows the types of polymers used, the type of drug used, the size of the particles, the encapsulation method employed, the method for its adherence to the HA coating and the distribution of the polymer with the drug.

Altogether 16 different types of polymer encapsulating drug were employed; 6 by Pera and 10 by Brace. The report also explains these findings in the discussion and concludes which methodology should be employed for the drug coating for pre-clinical trials.

Experimental

The drug encapsulation methods have been explained in detail in previous deliverables so the methodologies will not be explained in detail this section.

At Pera the sonication method was used to create microcapsules which had the polymer encapsulating the respective drug either gentamicin or alendronate.

Brace used methods of microencapsulation and film coating to coat the drug with a polymer. The polymers used for microencapsulation were TCP and HA, for film coating eutragit or carbopol were used. Again the two drug candidates used were gentamicin and alendronate.

Results

The table below shows the various methodologies and their results.

Method	Company	Polymers	Drug	Particle size	Encapsulation method	Adherence method to strip	Distribution
1	Pera	PLGA and PVA	Gentamicin sulfate	88% under 101µm	Sonication	Evaporation	Uneven, poor
1	Pera	PLGA and PVA	Alendronate dihydrate	86% under 101µm	Sonication	Evaporation	Uneven, poor
1	Pera	PLGA and PVA	Blank	100% under 84µm	Sonication	Evaporation	Uneven, poor
2	Pera	Phosphatidyl choline and cholesterol	Gentamicin sulfate	98% under 101µm	Sonication	Evaporation	Uneven, poor
2	Pera	Phosphatidyl choline and cholesterol	Alendronate dihydrate	100% under 84µm	Sonication	Evaporation	Uneven, poor
2	Pera	Phosphatidyl choline and cholesterol	Blank	99% under 101µm	Sonication	Evaporation	Uneven, poor
3	Brace	TCP and HA, 40 micron powders	Gentamicin sulfate	> 500 µm	Microencapsulation	N/A	N/A
3	Brace	TCP and HA, 40 micron powders	Alendronate dihydrate	> 500 µm	Microencapsulation	N/A	N/A
3	Brace	TCP and HA, 40 micron powders	Blank	> 500 µm	Microencapsulation	N/A	N/A
4	Brace	TCP and HA, 10 micron powders	Gentamicin sulfate	N/A	Microencapsulation	N/A	N/A
4	Brace	TCP and HA, 10 micron powders	Alendronate dihydrate	N/A	Microencapsulation	N/A	N/A
4	Brace	TCP and HA, 10 micron powders	Blank	N/A	Microencapsulation	N/A	N/A
5	Brace	Carbopol	Gentamicin sulfate	Film, no particle	Film coating	Film coated	Even, very good
5	Brace	Eutragit	Gentamicin sulfate	Film, no particle	Film coating	Film coated	Even, very good
5	Brace	Eutragit	Gentamicin sulfate at pH 2	Film, no particle	Film coating	Film coated	Even, very good
5	Brace	Eutragit	Alendronate dihydrate	Film, no particle	Film coating	Film coated	Even, very good

Discussion

The table illustrates the results of all the successful synthetic products developed by Brace and Pera in the research.

For this project, Pera used the procedure of sonication to allow microencapsulation of the drug with a polymer which was used in all six of the successful experiments. Adherence of the encapsulated drug to the hydroxyapatite on the metal was achieved by an evaporation technique of a required amount of the drug. Overall, the distribution of the polymer-encased drug on the hydroxyapatite was found to be uneven due to the rough surface.

For experiment 1, the polymers PLGA and PVA were used as the encapsulating polymer. In the sonication process they microencapsulated the gentamicin sulfate drug. The particle sizes of these were measured using zeta potential equipment and the results were that 88% of the particles were found to be less than 101 micrometers. The aim was to get a particle size of less than 150 micrometers.

For experiment 2, the polymers PLGA and PVA were used as the encapsulating polymer. In the sonication process they microencapsulated the alendornate dehydrate drug. The particle sizes of these were measured using zeta potential equipment and the results were that 86% of the particles were found to be less than 101 micrometers. The aim was to get a particle size of less than 150 micrometers.

For experiment 3, the polymers PLGA and PVA were used as the encapsulating polymer. In the sonication process no drug was used, in order to create blank particles so that the sizes can be compared with experiments 1 and 2. The particle sizes of these were measured using zeta potential equipment and the results were that 100% of the particles were found to be less than 84 micrometers. The particle sizes are considerably smaller than the sizes in experiments 1 and 2, this is because these particles contain no drug, so the diameters of the sphere particles are smaller. When a drug is used the sizes are larger in order to accommodate the drug being encapsulated.

For experiment 4, phosphatidyl choline and cholesterol were used as the encapsulating materials to create liposomes. In the sonication process the liposomes encapsulated the gentamicin sulfate drug. The particles sizes of these were measured using zeta potential equipment and the results were that 98% of the particles were found to be less than 101 micrometers. The aim was to get a particle size of less than 150 micrometers. In general, the particles sizes of the liposome encapsulating a drug were found to be smaller than using PLGA and PVA as the encapsulating material.

For experiment 5, phosphatidyl choline and cholesterol were used as the encapsulating materials to

create liposomes. In the sonication process the liposomes encapsulated the alendornate dehydrate drug. The particles sizes of these were measured using zeta potential equipment and the results were that 100% of the particles were found to be less than 84 micrometers. The aim was to get a particle size of less than 150 micrometers. In general, the particles sizes of the liposome encapsulating a drug were found to be smaller than using PLGA and PVA as the encapsulating material.

For experiment 6, phosphatidyl choline and cholesterol were used as the encapsulating materials to create liposomes. In the sonication process no drug was used, in order to create blank liposome particles so that the sizes can be compared with experiments 3 and 4. The particle sizes of these were measured using zeta potential equipment and the results were that 99% of the particles were found to be less than 101 micrometers. The particle sizes for blank liposomes do not vary much when compared to experiments 3 and 4, which contain a drug.

Brace used the two methods of microencapsulation and film coating to encapsulate the drug material.

For experiments 7, 8 and 9 tricalcium phosphate (TCP) and hydroxyapatite (HA) powders of 40 microns were used to microencapsulate gentamicin sulfate, alendronate dehydrate and no drug respectively. The particle size was measured and was found to be in excess of 500 micrometers, therefore was unsuitable for our requirements as particles sizes of less than 150 micrometers were required. Due to the large particle size the microencapsulated material was not taken further to adhere to the strip.

With experiments 7, 8, and 9 it was deduced that as a large powder size (40 microns) were used the final particle sizes were also great in positive correlation. Therefore it was predicted that if a smaller powder size was used then this would also reduce the final particle size.

The larger grain size powders for tricalcium phosphate (TCP) and hydroxyapatite (HA) were made smaller by jet-milling. Jet –milling was successful for both TCP and HA. Subsequently for experiments 10, 11 and 12 tricalcium phosphate (TCP) and hydroxyapatite (HA) powders of 10 microns were used to microencapsulate gentamicin sulfate, alendronate dehydrate and no drug respectively. The final particle size was measured and again was found to be unsuitable, therefore was unsuitable for our requirements as particles sizes of less than 150 micrometers were required. Due to the large particle size the microencapsulated material was not taken further to adhere to the strip.

As experiments 7, 8, 9, 10, 11 and 12 were found to unsuccessful; an alternative approach was adopted by Brace to coat the drug using suitable polymers. A film coating method was developed to coat the HA layer. The film coating would contain the drug in the polymer in a thin film sheet, so avoiding the issue of particle sizes. Adherence to the HA layer would be achieved by the film coating

sheet sticking to the surface of the HA.

For experiment 13, the polymer used was carbopol and was made into a film sheet with gentamicin sulfate as the drug. The adherence to the HA was successfully conducted and the distribution on the layer was even as a thin sheet was used.

For experiment 14, the polymer used was eutragit and was made into a film sheet with gentamicin sulfate as the drug. The adherence to the HA was successfully conducted and the distribution on the layer was even as a thin sheet was used. This shows that the process works well when two different types of polymers are used.

For experiment 15, the polymer used was eutragit and was made into a film sheet with gentamicin sulfate as the drug. For this experiment the drug was used at pH 2. The adherence to the HA was successfully conducted and the distribution on the layer was even as a thin sheet was used. This shows that the process works well even when the drug at a lower pH is used.

For experiment 16, the polymer used was eutragit and was made into a film sheet with alendronate dihydrate as the drug. The adherence to the HA was successfully conducted and the distribution on the layer was even as a thin sheet was used. This shows that the process also works well with alendronate dehydrate as the drug.

Conclusion

In conclusion, 16 different polymer encapsulating experiments were conducted showing a range of synthetic products/methods exploiting the method from Task 2.2.

The experiments at Pera were conducted using the sonication process to create micorspheres of PLGA and PVA or liposomes coating either of the two drugs; gentamicin sulfate or alendronate dehydrate. Blank polymer and liposome microspheres were also synthesised as a comparison for particle size. The majority of the particles synthesised were found to be less than 101 micrometers.

The initial experiments conducted by Brace using the microencapsulation method were found to be unsuccessful as the particle size was in excess of 500 micrometers. For microencapsulation TCP and HA were used to coat the drug materials. As this was unsuccessful a film method was developed, which would contain the drug in the polymer in a thin film. The polymers used were eutragit and carbopol. Film coating gave an even surface when coated on the HA.
The best method to take forward for coating implants was found to be the film coating as this has no particles involved and gave an even coating on the surface of the HA. The cytotoxicity tests Biomatech will able to confirm the safety of this material before an y pre-clinical work may commence.

Deliverable 6

Summary

The nature of this deliverable is under the category of 'prototype', so is given as a brief report showing several photographs of the prototypes of the drug coated stainless steel strip (pre-plasma sprayed) samples manufactured in the project ready for pre-clinical trials.

Introduction

This report shows the stainless steel strips that were plasma sprayed with hydroxyapatite were coated with either; carbopol and gentamicin, gentamicin slurry, gentamicin slurry at pH2 or eutragit and gentamicin, by Brace GmbH. In total 4 types of different drug coatings were conducted. The drug coating using either a polymer or slurry was successfully achieved. The slurry shows the multiple layering example of drug coating. Theses successfully coated prototypes are shown as photographs.

At Pera, the stainless steel strips (already plasma sprayed) were coated with either; PLGA encapsulating gentamicin, PLGA encapsulating alendronate, liposomes encapsulating gentamicin and liposomes encapsulating alendronate. In total 4 types of different drug coatings were conducted. The drug coating using either polymer to encapsulate the drug was successfully achieved. These successfully coated prototypes are shown as photographs.

Experimental

The drug coatings at Brace GmbH and Pera were both carried out using manual operation.

At Pera the sonication method was used to enable encapsulation of the drug and adherence to the strip was achieved by coating evenly on the hydroxyapatite surface and evaporation. At Brace the drug was coated using a film method. A film was used to coat the drug and the adherence to the strip was as a film. The slurry was coated in multiple film layers allowing time for the previous layer to dry before another could be added. At both institutes all the stainless steel strips were successfully coated.

Results

Several prototypes of the coating of drug bearing microcapsules onto HA surface and multiple layering have been made indicated by the following photographs:-

Figure 1: Carbopol and gentamicin coated stainless steel strip by Brace GmbH. The drug coating is a transparent gel on the surface of the hydroxyxapatite. The drug was film coated and adhered to the strip as a thin film. The coating was found to be even throughout.

Figure 2: Gentamicin slurry coated stainless steel strip by Brace GmbH. The drug coating is a transparent gel on the surface of the hydroxyxapatite. The drug was film coated and adhered to the strip as a thin film. Multiple layering of the slurry was conducted. The coating was found to be even throughout.

Figure 3: Gentamicin slurry at pH 2, coated stainless steel strip by Brace GmbH. The drug coating is a transparent gel on the surface of the hydroxyxapatite. The drug was film coated and adhered to the strip as a thin film. Multiple layering of the slurry was conducted. The coating was found to be even throughout.

Figure 4: Eutragit and gentamicin coated stainless steel strip by Brace GmbH. The drug coating is a transparent gel on the surface of the hydroxyxapatite. The drug was film coated and adhered to the strip as a thin film. The coating was found to be even throughout.

Figure 5: PLGA encapsulating gentamicin coated stainless steel strip by Pera. The drug coating is a transparent mixture on the surface of the hydroxyxapatite. The encapsulation formed microparticles of the polymer containing the drug, as the sonication method was used. The adherence to the strip was achieved by evaporation. The drug coating filled in the troughs on the hydroxyapatite surface as expected to there was an uneven coating.

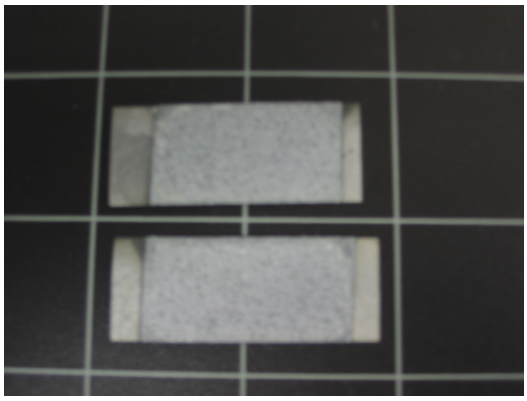


Figure 6: PLGA encapsulating alendronate coated stainless steel strip by Pera. The drug coating is a transparent mixture on the surface of the hydroxyxapatite. The encapsulation formed microparticles of the polymer containing the drug, as the sonication method was used. The adherence to the strip was achieved by evaporation. The drug coating filled in the troughs on the hydroxyapatite surface as expected to there was an uneven coating.

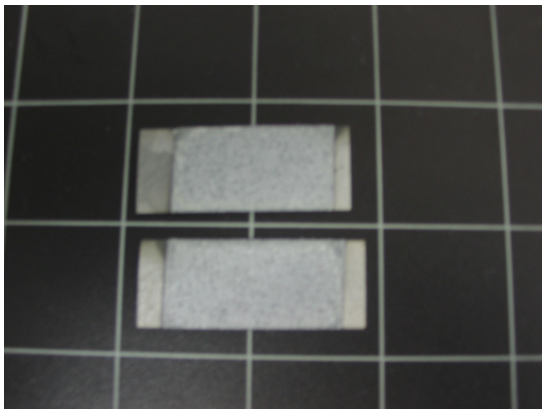
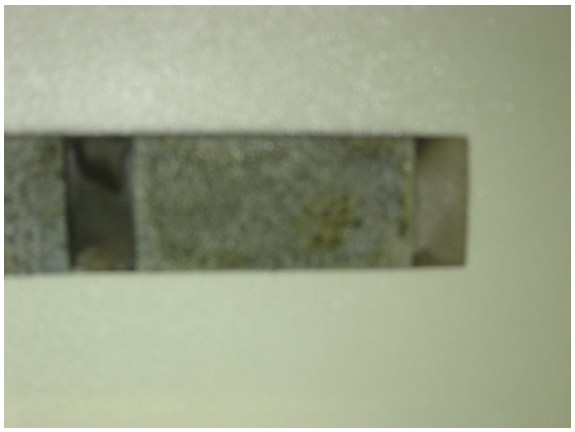


Figure 7: Liposomes encapsulating gentamicin coated stainless steel strip by Pera. The drug coating is a yellow mixture on the surface of the hydroxyxapatite. The yellow sections show the regions where there is a strong amount of liposomes containing drug material, other regions are more sparsely populated. The encapsulation formed microparticles of the polymer containing the drug, as the sonication method was used. The adherence to the strip was achieved by evaporation. The drug coating filled in the troughs on the hydroxyapatite surface as expected so there was an uneven coating.

Figure 7: Liposomes encapsulating alendronate coated stainless steel strip by Pera. The drug coating is a yellow mixture on the surface of the hydroxyxapatite. The yellow sections show the regions where there is a strong amount of liposomes containing drug material, other regions are more sparsely populated. The encapsulation formed microparticles of the polymer containing the drug, as the sonication method was used. The adherence to the strip was achieved by evaporation. The drug coating filled in the troughs on the hydroxyapatite surface as expected so there was an uneven coating.



Discussion

Overall successful coating of the hydroxyapatite layer on the stainless steel strip was achieved by both Pera and Brace. Both institutes used different approaches to achieve this.

Pera used the sonication method to enable drug encapsulation using either PLGA or liposomes as the polymer material. Both gentamicin and alendronate were successfully encapsulated with each of these polymers. The PLGA polymer gave a transparent material for coating, while the liposomes gave a yellow pigment as shown on the prototypes. These were then coated manually to achieve a coating on the surface of the implant. The adherence to the strip was achieved by evaporation. The drug coating filled in the troughs on the rugged hydroxyapatite surface as expected so there was an uneven coating.

At Brace GmbH the drug was encapsulated into a film, using; carbopol, eutragit polymers or a slurry. The film was then stretched over the hydroxyxapatite sections of the implant manually so that it adhered to the surface. As a film coating methodology was used the distribution was even over the strip.

As the stainless steel strips have been successfully coated they can now be used for the subsequent step, in pre-clinical trials. These are described in deliverables 8 and 9.

Conclusion

The aim of this deliverable was to show that a prototype for the drug microcapsules have been coated on a hydroxyapatite surface with also a multiple layering example. Both Brace and Pera have successfully coated stainless steel strips with hydroxyapatite using diffferent methodologies. At Brace coatings with carbopol and gentamicin, gentamicin slurry, gentamicin slurry at pH2 or eutragit and gentamicin were achieved. In total 4 types of different drug coatings were conducted. The drug coating using either a polymer or slurry was successfully achieved. The slurry shows the multiple layering example of drug coating. At Pera, the strips were coated with either; PLGA encapsulating gentamicin, PLGA encapsulating alendronate, liposomes encapsulating gentamicin and liposomes encapsulating alendronate. In total 4 types of different drug coatings were conducted. The drug coating using either polymer to encapsulate the drug was successfully achieved. These results show that the successful coating can now be used for the implants that will be used for pre-clinical work by Biomatech and Gothenburg University.

Deliverable 7

Summary

The nature of this deliverable is under the category of 'other', so is given as a brief report showing several photographs of the prototypes of the coated samples manufactured in the project for pre-clinical trials. There is information on the various implants to be coated, the method of plasma spraying used and microscopy photographs of the surface of the coated strips.

Introduction

This report shows the dimensions, type and material of the various implants manufactured in the project for pre-clinical work. The three types of medical grade metals used were; titanium, cobalt chrome or stainless steel. These metals were cut to the specific requirement needed for the study. Specific regions of these implants were then plasma sprayed (explained later) using titanium (if titanium metal was not used) and then hydroxyapatite. The surfaces of these plasma sprayed implants were then analysed using microscopy.

Experimental

Implants Design

For the pre-clinical trials to be performed at Biomatech and Gothenburg University a range of prototype implants are required, these are;

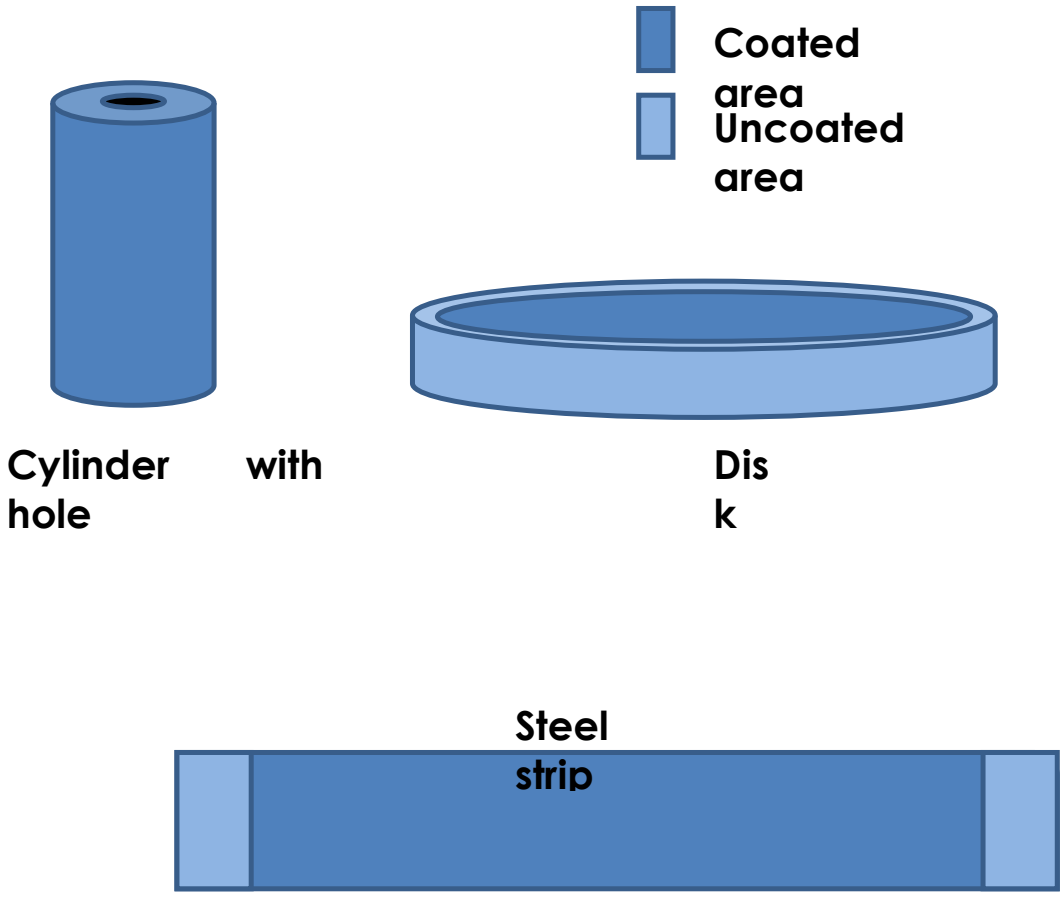
- 90 Titanium disks (10 mm diameter x 1 mm thickness)
- 84 Cobalt chrome disks (10 mm diameter x 1 mm thickness)
- 42 Cobalt chrome cylinders (3.75 mm diameter x 4 mm height)

For the preliminary chemical synthesis metal strips coated with hydroxyapatite were required.

- 35 Stainless steel strips coated with hydroxyapatite (20 mm width x 100 mm length)
- 35 Cobalt chrome strips coated with hydroxyapatite (20 mm width x 100 mm length)

For all the prototype implants mentioned above medical grade metals were used. The size and dimensions of the implants were calculated to be suitable for the rabbit model to be used in the pre-clinical work.

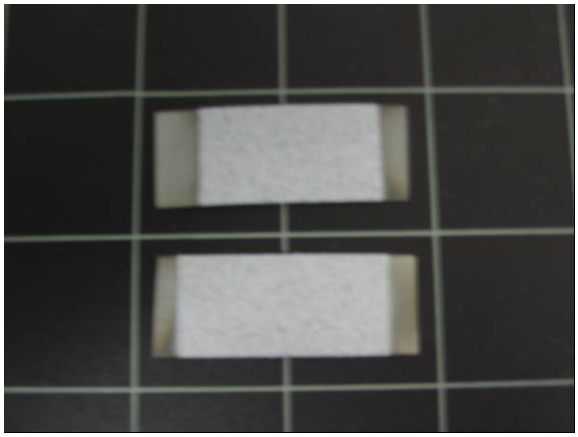
Figure 1 Implants required for pre-clinical work



Metal implants manufactured by Pera

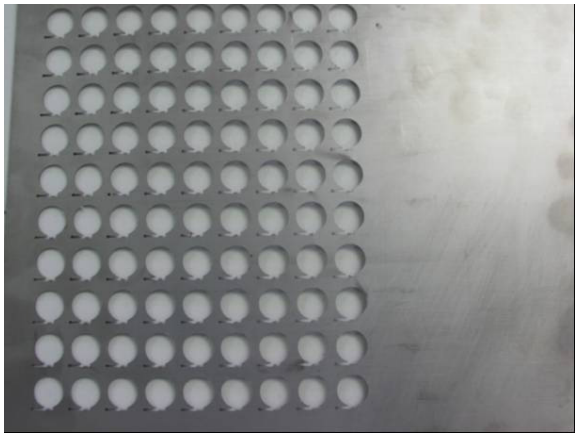
The medical grade stainless steel strips were machined and cut at Pera. These were subsequently sent to Medicoat for plasma spraying with titanium and hydroxyapatite as shown below.

Figure 2 Hydroxyapatite coated stainless steel strip



Titanium disks were also machined from a sheet of medical grade titanium, to the required specification using water-jet cutting methodology. These 90 disks have been subsequently sent to Medicoat for the plasma spraying process.

Figure 3 Titanium sheet after cutting of disks



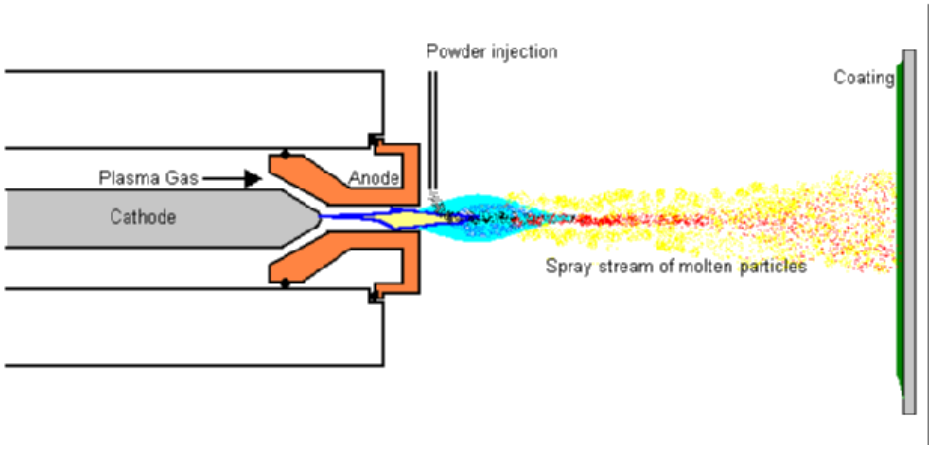
Fundamentals of Plasma spraying

Plasma spraying of the stainless steel strips was completed at Medicoat. The strips were coated with hydroxyapatite using plasma spraying. This methodology will also be adopted for the plasma spraying of the final pre-clinical implants.

The hydroxyapatite and tricalciumphosphate were supplied by Teknimed (exclusive supplier for the project). These powders have been tested for purity and acceptability for use in medical implants. This has been used for successful multi-layering of samples for pre-clinical trials.

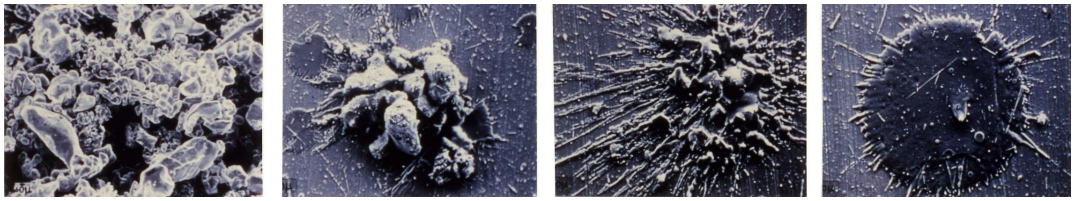
Powder particles, fed into a plasma jet, are accelerated and melted during flying. The coating is formed by the successive layers of liquid droplets flatten on impact.

Figure 4 Schematic diagram of Plasma Spraying



The molten state of the injected particles before impinging on the substrate surface determines the coating structure. Functional coatings by selected particle size distribution in relationship to adapted plasma energy and enthalpy.

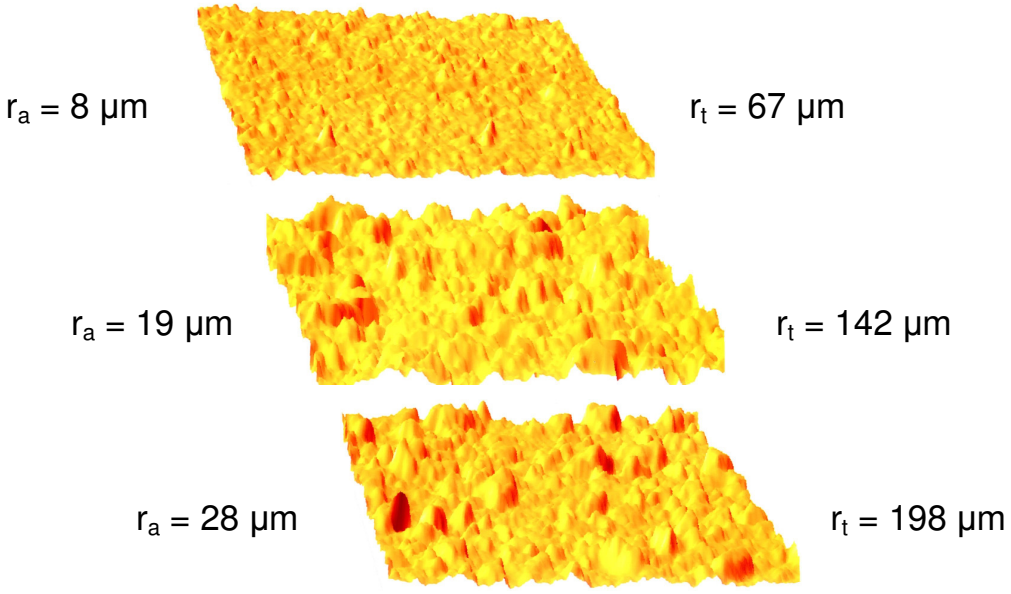
Figure 5 Images of plasma sprayed surface



Powder Degree of particle melting

By altering the variables for plasma spraying the morphology of the coatings could be altered to enable a different surface roughness of the implants. The roughness of the surface is important to allow a textured base for the drug coating to adhere to. Surface roughness measurements have been taken for this study and are shown in Deliverable 5.

Figure 6 Morphology of coatings



Vacuum plasma spraying (VPS) is a technology for etching and surface modification to create porous layers with high reproducibility and for cleaning and surface engineering of plastics, rubbers and natural fibres as well as for replacing CFCs for cleaning metal components. This surface engineering can improve properties such as frictional behaviour, heat resistance, surface electrical conductivity, lubricity, cohesive strength of films, or dielectric constant, or it can make materials hydrophilic or hydrophobic.

Vacuum plasma treatment is a process typically operating at 39–120 ℃ to avoid thermal damage. The process can induce non-thermally activated surface reactions, causing surface changes which cannot occur with molecular chemistries at atmospheric pressure.

Plasma processing is done in a controlled environment inside a sealed chamber at a medium vacuum, around 13–65 Pa. The gas or mixture of gases is energised by an electrical field from DC to microwave frequencies, typically 1–500 W at 50 V. The treated components are usually electrically isolated. The volatile plasma by-products are evacuated from the chamber by the vacuum pump, and if necessary can be neutralised in an exhaust scrubber.

In contrast to molecular chemistry, plasmas employ:

- Molecular, atomic, metastable and free radical species for chemical effects.
- Positive ions and electrons for kinetic effects.

Plasma also generates electromagnetic radiation in the form of vacuum UV photons to penetrate bulk polymers to a depth of about 10 µm. This can cause chain scissions and cross-linking.

Plasmas affect materials at an atomic level. Techniques like X-ray photoelectron spectroscopy and scanning electron microscopy are used for surface analysis to identify the processes required and to judge their effects. As a simple indication of surface energy, and hence adhesion or wetability, often a water droplet contact angle test is used. The lower the contact angle, the higher the surface energy and more hydrophilic the material is.

At higher energies ionisation tends to occur more than chemical dissociations. In a typical reactive gas, 1 in 100 molecules form free radicals whereas only 1 in 106 ionises. The predominant effect here is the forming of free radicals. Ionic effects can predominate with selection of process parameters and if necessary the use of noble gases.

Figure 7 C - VPS coating process

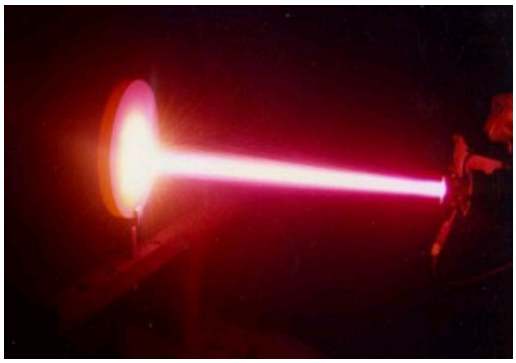


Figure 8 HF - VPS coating process

The equipment used for plasma spraying at Medicoat is used thermal spray equipments of the newest technology.

The coating systems were designed for optimized production economy, highest coating quality and reproducibility for the process cycle. The modern PC / PLC control unit offered an easy operation of

the equipment, data handling and storage and the integration of additional features.

Figure 9 C - VPS plant

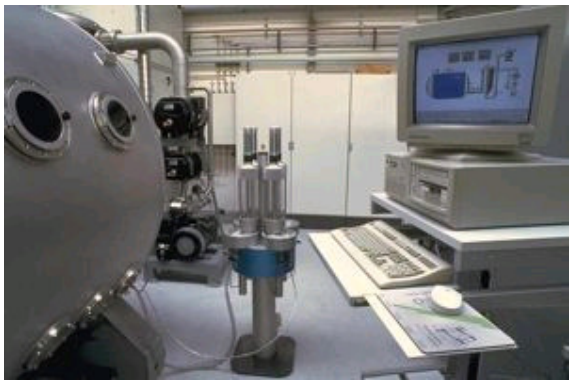


Figure 10 C / HF - VPS system

Results

Successful plasma spraying was achieved on the implants for pre-clinical trials. As this has been accomplished then drug coating on this surface will be possible to go to the next step for pre-clinical trials. These are illustrated on the microscopy photographs below.

The microscopy photographs below indicate that the plasma spraying is not even and smooth. The plasma spraying is jagged throughout the implant having peaks and valleys. Therefore any drug coating is likely to fill-in the valley regions.

Figure 11 Side-view of the plasma sprayed surface illustrating the hydroxyapatite coated (dark region)

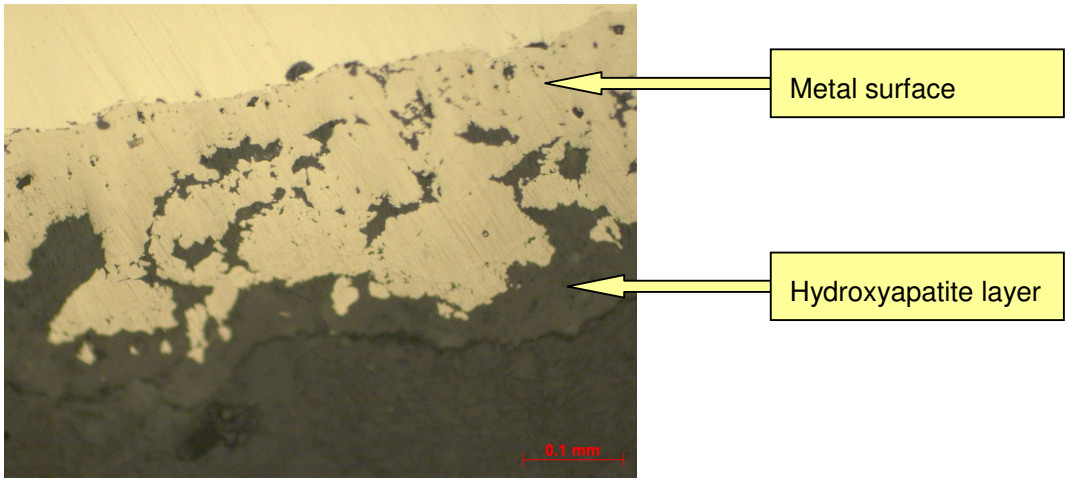
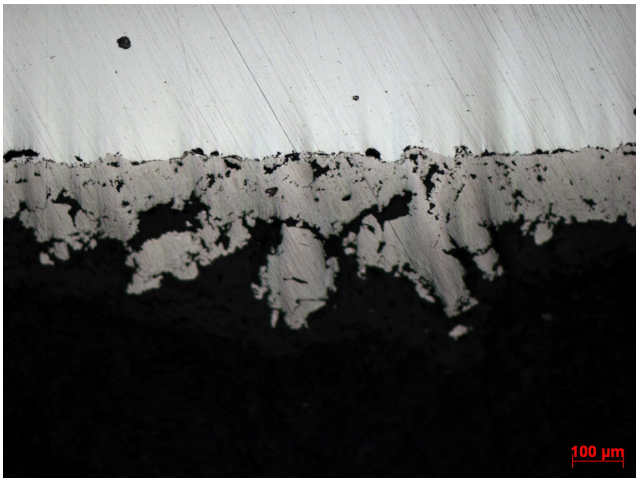


Figure 12 Side-view of the plasma sprayed surface illustrating the hydroxyapatite coated (dark region)



The overhead-view of the implants was also investigated showing a rough coating of hydroxyapatite on the metal surfaces.

Figure 13 Overhead -view of the plasma sprayed surface illustrating hydroxyapatite coating

Discussion

As plasma spraying has been successfully achieved for the implants this allows progression to the subsequent stage which is encapsulation of the drug using a polymer, coating the surface of the implant. For the next stage, the drug coated disks and strips will be used by Biomatech in their research for its biocompatibility by *in vitro* work. The drug coated cylinders will be used by Gothenburg University for their *in vivo* studies on the rabbit model.

Conclusion

This deliverable is under the category of 'other', therefore is shown as a brief report showing several photographs of the prototypes of the coated samples manufactured in the project for pre-clinical trials. This report illustrated the different types of implants that were needed to be plasma coated, how the plasma coating process was achieved, microscopy photographs indicating the surface roughness and the use of the coated billets for the pre-clinical trials.

Deliverable 8 REPORT BY BIOMATECH

Cytotoxicity Study Using the ISO 10993 Standard with Extract on; the PLGA and Gentamicin Coated Discs (Substraight is CoCr Ti Ha), and with Extract on the HA Slurry Coated Discs CC/Ti/HA, 10 mg Hydroxyl Apatite

SUMMARY

An *In vitro* biocompatibility test on; the PLGA and Gentamicine coated discs (substraght is CoCr Ti Ha), and on the HA Slurry coated discs CC/Ti/HA, 10 mg Hydroxyl Apatite, batch 1450-0507/200800245 was conducted to evaluate the potential for cytotoxicity. This study was conducted according to the requirements of the ISO 10993 standard : Biological Evaluation of Medical Devices, Part 5 (1999) : Tests for *in vitro* cytotoxicity. An extract of the test article was prepared as follows :

- Extraction vehicle : Minimum Essential Medium Eagle 1X (EMEM 1X) supplemented with L-glutamine 1 % (v/v), foetal bovine serum 10 % (v/v) and antibiotics (penicillin – streptomycin 2 % (v/v))
- Temperature : 37 +/- 1°C
- Duration : 24-26 hours with agitation
- Ratio test article/vehicle : 3 cm²/mL

This test extract was diluted in supplemented EMEM 1X and placed onto triplicate confluent monolayers of L-929 mouse fibroblast cells pure and at the 50 %, 25 % and 5 % dilutions. Separate monolayers were prepared for triplicate negative and positive controls and their dilutions. After incubating at 37 ± 1°C in 5 ± 1 % CO_2 for 24-26 hours, the cell cultures were stained by a neutral red solution and examined microscopically (at least 100X) to determine cell morphology. The dye was then extracted from the cultures and optical density was measured at 550 nm.

Under the conditions of this study, the extract of the test article for the PLGA and Gentamicine coated discs (substraght is CoCr Ti Ha) showed the following results:

Concentration	Interpretation	Percentage of reduction of the cells density compared to the negative control
pure	Moderately cytotoxic	15.9 %*
50 %	Not cytotoxic	-11.7 %
25 %	Not cytotoxic	-12.3 %
5 %	Not cytotoxic	-12.0 %

* No percentage of reduction of the cells density because of the red neutral particles on the cells

The negative and positive controls performed as anticipated.

Under the conditions of this study, the extract of the test article for the HA Slurry coated discs CC/Ti/HA, 10 mg Hydroxyl Apatite showed the following results:

Concentration	Interpretation	Percentage of reduction of the cells density compared to the negative control
pure	Not cytotoxic	-14.8 %*
50 %	Not cytotoxic	1.8 %
25 %	Not cytotoxic	0.3 %

* : Slight cell proliferation compared to the negative control.

The negative and positive controls performed as anticipated.

INTRODUCTION

The test articles identified below were subjected to an *in vitro* cytotoxicity study for biocompatibility based on the requirement of the ISO 10993 standard: Biological Evaluation of Medical Devices, Part 5 (1999): Tests for *in vitro* cytotoxicity. The purpose of the study was to determine whether leachables extracted from the material would cause cytotoxicity.

MATERIALS

The test article provided by the Sponsor was identified and handled as follows :

Name of the test article 1	:	PLGA and Gentamicin coated discs (substraight is CoCr Ti HA)
Name of the test article 2	:	HA Slurry coated discs CC/Ti/HA, 10 mg Hydroxyl Apatite
Sterilization process / dose	:	Gamma irradiated (25-35 kGy)
Storage conditions	:	Room temperature (+ 15°C / + 25°C)
Quantity of test used article	:	3 and 2 pieces respectively
Extraction conditions	:	
- Extraction vehicle	:	Minimum Essential Medium Eagle 1X (EMEM 1X) supplemented with L-glutamine 1 % (v/v), foetal bovine serum and antibiotics ((penicillin – streptomycin 2 % (v/v))
- Duration	:	24-26 hours with agitation
- Ratio test article/vehicle	:	3 cm²/mL
- Temperature	:	37 +/- 1°C
- Test article preparation 1	:	4.71 cm² of the test article were covered with 1.6 mL of supplemented EMEM 1X. A single preparation was subjected to the extraction conditions previously described. The pure extract

		was used and at 50%, 25% and 5% dilutions, after dilution in supplemented MEM.
- Test article preparation 2	:	3.2 cm² of the test article were covered with 1.1 mL of supplemented EMEM 1X. A single preparation was subjected to the extraction conditions previously described. The pure extract was used pure and at 50% and 25% dilutions, after dilution in supplemented MEM. The coating of the discs was partialy dissolved in the extraction vehicle.
- Aspect of the extract/ conditions of use	:	After extraction, the extract was cooled to room temperature, vigorously shaken, centrifuged at 200 g during 5 minutes in order to eliminate the particles and used immediately.
Negative control	:	HDPE (high density polyethylene, ratio of 3 cm²/mL) was used as the negative control. A single preparation was subjected to the extraction conditions previously described, and diluted like the test article extract.
Positive control	:	6.4 g/L phenol in supplemented EMEM 1X and diluted like the test article extract.
Test article disposition	:	Unused test article will be returned to the Sponsor after the end of the study.

METHODS

Test System Management and Justification

Mammalian cell culture monolayers, L929, mouse fibroblast (ATCC CCL1, NCTC clone 929, of strain L, or equivalent source) were used. *In vitro* mammalian cell culture studies have been used historically to evaluate cytotoxicity of biomaterials and medical devices, and L929 cells are recommended by the ISO 10993-5 Standard.

These cells were transferred and propagated at 37 ± 1°C in a gaseous environment of 5 ± 1 % carbon dioxide (CO_2) in an open flask containing Minimum Essential Medium (EMEM 1X) supplemented with 10 % foetal bovine serum (v/v), 1 % L-glutamine (v/v) and an appropriate concentration of antibiotics ((penicillin – streptomycin 2 % (v/v)). For this study, 96-well plates were seeded, labelled with passage number and date, and incubated at 37 ± 1°C in 5 ± 1 % CO_2 to obtain confluent monolayers of cells prior to use. Aseptic procedures were used in the handling of the cell cultures following approved BIOMATECH Procedures (I-BIO 080).

Experimental procedure

Triplicate culture wells, which contained a confluent cell monolayer were selected. The growth medium was replaced with 200 µL of the test article extract (pure) or its dilutions (50 %, 25 % and 5 %) in supplemented EMEM 1X. Similarly, triplicate culture wells were prepared for each negative and positive control replacing the growth medium in each culture

by 200 µL of the controls. Each well was labelled indicating its content and replicate number. The test and controls wells were incubated at 37 ± 1°C in 5 ± 1 % CO_2 for 24-26 hours. Following incubation, the cultures were stained by a neutral red solution and examined microscopically (at least 100X) to determine cell morphology. The cells were then fixed, the dye extracted from the cultures and the optical density (OD) was measured at 550 nm.

Qualitative scoring for cytotoxicity was based on the following criteria :

Table 1 : Scoring for Cytotoxicity

RESPONSE INDEX	CULTURES DESCRIPTION	INTERPRETATION
0	Intact cells, stained, confluent layer	Not cytotoxic
1	Stained cells with a slight decrease in the cell density or slight morphological alterations	Slightly cytotoxic
2	Stained cells with a large decrease in the cell density or severe morphological alterations	Moderately cytotoxic
3	Cell lysis or complete absence of neutral red incorporation	Severely cytotoxic

The quantitative evaluation of the cytotoxicity was performed measuring optical density in the wells of the test article extract, negative and positive controls. A mean score was calculated and compared to the negative control mean. A reduction percentage compared to the negative control was calculated.

For the suitability of the system to be confirmed, the negative control must be not cytotoxic. The pure positive control must have shown a severe toxicity, as compared to the negative control. The test would be repeated if the controls did not perform as anticipated and/or if one of the three test wells did not yield the same conclusion.

RESULTS

The scores and optical densities obtained were as follows:

Table 2 : Qualitative and quantitative results PLGA and Gentamicin coated discs (substraight is CoCr Ti HA)

RESPONSE INDEX	OD	MEAN OD	% OF NEGATIVE CONTROL	% OF REDUCTION

Test article (pure)	2 2 2	1.515 1.579 0.627	1.240	84.1%	**15.9%**
Positive control (pure)	3 3 3	0.482 0.545 0.433	0.487	33.0%	67.0%
Negative control (pure)	0 0 0	1.499 1.662 1.266	1.476	100.0%	0.0%
Test article (50%)	0 0 0	1.619 1.706 1.620	1.648	111.7%	**-11.7%**
Test article (25%)	0 0 0	1.666 1.592 1.715	1.658	112.3%	**-12.3%**
Test article (5%)	0 0 0	1.673 1.650 1.637	1.653	112.0%	**-12.0%**

Table 3: Qualitative and quantitative results HA Slurry coated discs CC/Ti/HA, 10 mg Hydroxyl Apatite

	RESPONSE INDEX	OD	MEAN OD	% OF NEGATIVE CONTROL	% OF REDUCTION
Test article (pure)	0 0 0	1.992 1.764 1.980	1.912	114.8%	**-14.8%**
Positive control (pure)	3 3 3	0.079 0.072 0.074	0.075	4.5%	95.5%
Negative control (pure)	0 0 0	1.797 1.599 1.600	1.665	100.0%	0.0%
Test article (50%)	0 0 0	1.814 1.483 1.607	1.635	98.2%	**1.8%**
	0	1.584			

Test article (25%)	0 0	1.513 1.886	1.661	99.7%	**0.3%**

Results and conclusions apply only to the test article tested. No further evaluation of these results is made by BIOMATECH. Any extrapolation of these data to other samples is the responsibility of the Sponsor. All procedures were conducted in conformance with NF EN ISO/CEI 17025 (September 2005) Quality Standards and the program n° 130-1 from the COFRAC (French Committee for Accreditation) « Bio cytotoxicity « in vitro » determination assays of materials and medical devices » - Tests for in vitro cytotoxicity - code CV.10.

ADHERENCE TO PROTOCOL / STANDARD

The positive control was not conformed to Biomatech specifications. But the results were accepted because the positive control still showed a severe cytotoxicity with a response index of 3 at the qualitative evaluation.

CONCLUSION

Under the conditions of this study, the extract of the test article showed the following results for PLGA and Gentamicin coated discs (substraight is CoCr Ti HA):

Concentration	Interpretation	Percentage of reduction
pure	Moderately cytotoxic	15.9 %*
50 %	Not cytotoxic	-11.7 %
25 %	Not cytotoxic	-12.3 %
5 %	Not cytotoxic	-12.0 %

* No percentage of reduction of the cells density because of the red neutral particles on the cells

The negative and positive controls performed as anticipated.

Under the conditions of this study, the extract of the test article showed the following results for HA Slurry coated discs CC/Ti/HA, 10 mg Hydroxyl Apatite:

Concentration	Interpretation	Percentage of reduction of the cells density compared to the negative control
pure	Not cytotoxic	-14.8 %*
50 %	Not cytotoxic	1.8 %
25 %	Not cytotoxic	0.3 %

* : Slight cell proliferation compared to the negative control.

The negative and positive controls performed as anticipated.

DATA RECORDING AND ARCHIVING

All study file data including raw data (with blocks and slides of histological specimens), protocols, reports and ancillary documents submitted by the Sponsor, at the time the order was placed will be archived at BIOMATECH for 5 years from mailing of the final report. After the 5 years period, these items will be subject to destruction unless specific and written instruction to return them to the Sponsor is provided to BIOMATECH. Sponsor confidentiality will be protected at all times

REPORT BY GOTHENBURG UNIVERSITY

PROJECT DENDRITES: Effects of selected encapsulated drugs on bone-promoting cells

Background

Prosthetic implants are well-established treatment for osteoarthritic and trauma patients and account for over 1,4 million operations annually across Europe. However, postoperative infections are known to occur resulting approximately 50000 cases of deep bone and 193000 cases of superficial infection. A number of implant improvements have been done in order to overcome the prosthetic implant loosening: the improvement of bone cement, hydroxyapatite coating of the metal surface and the addition of a phospholipid-monomer as a protecting layer. However, despite advances loosening remains a problem and there are other complications of infection and inflammation that continue to cause difficulty for patients and medical services.
Poor implant stability leads to the loss of either biological or cement fixation and results in wear, pain, loss of function and even fracture of the implant each of which could necessitate further surgery. The need for antibiotic therapy both orally and intravenously ranges from a period of 6 weeks to 6 months for the treatment of prosthetic infections. The appearance of antibiotic resistant bacteria and the formation of the biofilm on the implant surface demands the antibiotic concentration at the infected site 100-1000 times more than minimum inhibitory concentration (MIC). Thus, there is a need of the implant surface modification towards local drug delivery.

The purpose of the study is to evaluate human osteoblasts (MG63) number, viability and function in vitro on candidate materials compared to uncoated controls. The results will be presented in the FINAL REPORT 090801.

Materials and Methods

Implants

CoCr discs with diameter of 10 mm and 1 mm thickness were coated with Ti and hydroxyapatite. Thereafter different amount of Gentamicin (2 or 10mg) were encapsulated in HA slurry and attached to the surfaces as a coating. The control were uncoated CoCr with Ti and HA. The discs will be sterilised, packed and distributed to the laboratory from the partners of the EU Dendrites consortium during Spring 2009.

MG63 Cell Line

The human osteoblast-like (osteosarcoma) cell line MG-63 (ATCC, CRL-1427, US) will be used. MG-63 cells exhibit mature osteoblastic traits like differentiation, proliferation and production of membrane-associated alkaline phosphatase (ALP), bone matrix molecule collagen I (Coll I) and a variety of non-collagenous proteins, such as osteocalcin (OC).

The cells will be cultured in 75 cm2 tissue culture flasks (Falcon, US) in Dulbecco´s modified Eagle medium (DMEM, Gibco, UK) (including HEPES in the material experiments), containing 10% heat-activated fetal bovine serum (FBS, Gibco, UK) and 1% of penicillin G sodium/streptomycin sulphate (PEST) and fungizone (Gibco, UK), at 37□C in an atmosphere of 5% CO2 and 95% humidity.

When the cells reach confluence they will be gently washed with Hank´s balanced salt solution (HBSS without Ca2+ and Mg 2+) for 30 s, trypsinized 81,5ml 0,52mM trypsin-EDTA, Sigma) for 5-10 minutes, aspired with (approximately 10ml) complete growth medium and aliquots were added to 3 new flasks with complete growth medium (to a total volyme of 15ml per flask). The medium will be exchanged three times a week. At passage 6 the cells will be trypsinized and seeded on the material surfaces in 24-wells plates at a concentrations 7500 cells/well. Cells will be harvested after 1, 3, 14 and 21 days. During the experiments the medium will be exchanged three times a week.

Cell number

Cell amounts in association with the surfaces and surrounding medium will be determined by a NucleoCounter® system (ChemoMetec A/S, Denmark). Briefly, cells will be treated with lysis buffer and stabilizing buffer (provided with the system). Lysed samples will be loaded in a NucleoCassette™ precoated with fluorescent propidium iodide that stains the cell nuclei, and will be then quantified in the NucleoCounter®.

Cell viability

Cell viability will be determined by measuring lactate dehydrogenase (LDH), a marker of cell membrane injury, in the culture medium using a spectrophotometric evaluation of LDH mediated conversion of pyruvic acid to lactic acid (C-Laboratory, Sahlgrenska University Hospital, Göteborg, Sweden).

mRNA determination by qPCR

Discs with attached osteoblasts will be placed in RNAlater® solution and stored at -80 □C until analysis. Total RNA will be extracted using RNAeasy® Microkit and Minikit (QIAGENE) respectively. Reverse transcription will be performed. The forward (FW) and reverse (RV) primers were designed and optimized using software for alkaline phosphatase (ALP), osteocalcin (OC), collagen I and 18 S (cell number). The PCR mixture, containing cDNA template, the forward and reverse primers, and SYBR Green Mix, will be amplified using Eppendorf PCR instrument.

The PCR conditions will be adjusted according to the protocol. Each sample will be tested in duplicates, and the threshold cycle (Ct) values will be averaged from each reaction. The quantification strategy will be based on comparing two genes from two sample using the formula $k*1.9\Delta ct$.

Statistics

Student t test will be performed.

Results

Will be presented in the Final Report 090801

Deliverable 9 REPORT BY BIOMATECH

Cytotoxicity Study Using the ISO 10993 Standard with Extract on the HA Slurry Coated Discs; CoCr Ti HA (Control), with 20 mg Gentamicin Loading, with 40 mg Gentamicin Loading, with 80 mg Gentamicin Loading.

SUMMARY

An *In vitro* biocompatibility test on; the CoCr Ti Ha (control), with 20 mg Gentamicin Loading, with 40 mg Gentamicin Loading, with 80 mg Gentamicin Loading were conducted to evaluate the potential for cytotoxicity. This study was conducted according to the requirements of the ISO 10993 standard : Biological Evaluation of Medical Devices, Part 5 (1999) : Tests for *in vitro* cytotoxicity. An extract of the test article was prepared as follows:

- Extraction vehicle : Minimum Essential Medium Eagle 1X (EMEM 1X) supplemented with L-glutamine 1 % (v/v), foetal bovine serum 10 % (v/v) and antibiotics (penicillin – streptomycin 2 % (v/v))
- Temperature : 37+/-1°C
- Duration : 24-26 hours with agitation
- Ratio test article/vehicle : 3 cm^2/mL

This test extract was diluted in supplemented EMEM 1X and placed onto triplicate confluent monolayers of L-929 mouse fibroblast cells pure and at the 50 %, 25 % and 5 % dilutions. Separate monolayers were prepared for triplicate negative and positive controls and their dilutions. After incubating at 37 ± 1°C in 5 ± 1 % CO_2 for 24-26 hours, the cell cultures were stained by a neutral red solution and examined microscopically (at least 100X) to determine cell morphology. The dye was then extracted from the cultures and optical density was measured at 550 nm.

Under the conditions of this study, the extract of the test article showed the following results for the CoCr Ti Ha (control):

Concentration	Interpretation	Percentage of reduction of the cells density compared to the negative control
pure	Slightly cytotoxic	6.7 %
50 %	Not cytotoxic	-3.8 %
25 %	Not cytotoxic	4.9 %
5 %	Not cytotoxic	0.6 %

The negative and positive controls performed as anticipated.

Under the conditions of this study, the extract of the test article showed the following results for 20 mg Gentamicin Loading:

Concentration	Interpretation	Percentage of reduction of the cells density compared to the negative control
pure	No determinate because too much particles on the cells	-1.0 %
50 %	Moderately cytotoxic	-9.7 %*
25 %	Not cytotoxic	-1.1 %
5 %	Not cytotoxic	-5.8 %

* No percentage of reduction of the cell density because of the red neutral particles on the cells

The negative and positive controls performed as anticipated.

Under the conditions of this study, the extract of the test article showed the following results for 40 mg Gentamicin Loading:

Concentration	Interpretation	Percentage of reduction of the cells density compared to the negative control
pure	Severely cytotoxic	88.9 %
50 %	Moderately cytotoxic	52.6 %
25 %	Moderately cytotoxic	18.3 %
5 %	Not cytotoxic	0.8 %

The negative and positive controls performed as anticipated.

Under the conditions of this study, the extract of the test article showed the following results for 80 mg Gentamicin Loading:

Concentration	Interpretation	Percentage of reduction of the cells density compared to the negative control
pure	Severely cytotoxic	92.2 %
50 %	Severely cytotoxic	93.9 %
25 %	Moderately cytotoxic	54.7 %
5 %	Not cytotoxic	-12.2 %

The negative and positive controls performed as anticipated.

INTRODUCTION

The test article identified below was subjected to an *in vitro* cytotoxicity study for biocompatibility based on the requirement of the ISO 10993 standard : Biological Evaluation of Medical Devices, Part 5 (1999): Tests for *in vitro* cytotoxicity. The purpose of the study was to determine whether leachables extracted from the material would cause cytotoxicity.

MATERIALS

The test article provided by the Sponsor was identified and handled as follows :

Name of the test article 1	:	CoCr Ti Ha (control) (CoCr discs with Ti, Ha plasma sprayed coating)
Name of the test article 2	:	HA slurry coated discs with 20 mg gentamicin loading (substraght is CoCr Ti Ha) (CoCr discs with Ti, Ha plasma sprayed coating with additional solution driped coating of Ha slurry with 20 mg gentamicin loading)
Name of the test article 3	:	HA slurry coated discs with 20 mg gentamicin loading (substraght is CoCr Ti Ha) (CoCr discs with Ti, Ha plasma sprayed coating with additional solution driped coating of Ha slurry with 40 mg gentamicin loading)
Name of the test article 4	:	HA slurry coated discs with 20 mg gentamicin loading (substraght is CoCr Ti Ha) (CoCr discs with Ti, Ha plasma sprayed coating with additional solution driped coating of Ha slurry with 80 mg gentamicin loading)
Sterilization process / dose	:	Gamma irradiated
Storage conditions	:	Room temperature (+ 15°C / + 25°C)
Quantity of test used article	:	3 pieces
Extraction conditions	:	
- Extraction vehicle	:	Minimum Essential Medium Eagle 1X (EMEM 1X) supplemented with L-glutamine 1 % (v/v), foetal bovine serum and antibiotics ((penicillin – streptomycin 2 % (v/v))
- Duration	:	24-26 hours with agitation
- Ratio test article/vehicle	:	3 cm²/mL
- Temperature	:	37 +/- 1°C
- Test article preparation	:	4.71 cm² of the test article were covered with 1.6 mL of supplemented EMEM 1X. A single preparation was subjected to the extraction conditions previously described. The pure extract was used and at 50%, 25% and 5% dilutions, after dilution in supplemented MEM.
- Aspect of the extract/ conditions of use	:	After extraction, the extract was cooled to room temperature, vigorously shaken, centrifuged at 200 g during 5 minutes in order to eliminate the particles and used immediately.

Negative control	:	HDPE (high density polyethylene, ratio of 3 cm²/mL) was used as the negative control. A single preparation was subjected to the extraction conditions previously described, and diluted like the test article extract.
Positive control	:	6.4 g/L phenol in supplemented EMEM 1X and diluted like the test article extract.
Test article disposition	:	Unused test article will be returned to the Sponsor after the end of the study.

METHODS

Test System Management and Justification

Mammalian cell culture monolayers, L929, mouse fibroblast (ATCC CCL1, NCTC clone 929, of strain L, or equivalent source) were used. *In vitro* mammalian cell culture studies have been used historically to evaluate cytotoxicity of biomaterials and medical devices, and L929 cells are recommended by the ISO 10993-5 Standard.

These cells were transferred and propagated at 37 ± 1°C in a gaseous environment of 5 ± 1 % carbon dioxide (CO_2) in an open flask containing Minimum Essential Medium (EMEM 1X) supplemented with 10 % foetal bovine serum (v/v), 1 % L-glutamine (v/v) and an appropriate concentration of antibiotics ((penicillin – streptomycin 2 % (v/v)). For this study, 96-well plates were seeded, labelled with passage number and date, and incubated at 37 ± 1°C in 5 ± 1 % CO_2 to obtain confluent monolayers of cells prior to use. Aseptic procedures were used in the handling of the cell cultures following approved BIOMATECH Procedures (I-BIO 080).

Experimental procedure

Triplicate culture wells, which contained a confluent cell monolayer were selected. The growth medium was replaced with 200 µL of the test article extract (pure) or its dilutions (50 %, 25 % and 5 %) in supplemented EMEM 1X. Similarly, triplicate culture wells were prepared for each negative and positive control replacing the growth medium in each culture by 200 µL of the controls. Each well was labelled indicating its content and replicate number. The test and controls wells were incubated at 37 ± 1°C in 5 ± 1 % CO_2 for 24-26 hours. Following incubation, the cultures were stained by a neutral red solution and examined microscopically (at least 100X) to determine cell morphology. The cells were then fixed, the dye extracted from the cultures and the optical density (OD) was measured at 550 nm.

Qualitative scoring for cytotoxicity was based on the following criteria :

Table: Scoring for Cytotoxicity

RESPONSE	CULTURES DESCRIPTION	INTERPRETATION

INDEX		
0	Intact cells, stained, confluent layer	Not cytotoxic
1	Stained cells with a slight decrease in the cell density or slight morphological alterations	Slightly cytotoxic
2	Stained cells with a large decrease in the cell density or severe morphological alterations	Moderately cytotoxic
3	Cell lysis or complete absence of neutral red incorporation	Severely cytotoxic

The quantitative evaluation of the cytotoxicity was performed measuring optical density in the wells of the test article extract, negative and positive controls. A mean score was calculated and compared to the negative control mean. A reduction percentage compared to the negative control was calculated.
For the suitability of the system to be confirmed, the negative control must be not cytotoxic. The pure positive control must have shown a severe toxicity, as compared to the negative control.
The test would be repeated if the controls did not perform as anticipated and/or if one of the three test wells did not yield the same conclusion.

RESULTS

The scores and optical densities obtained were as follows :

Table: Qualitative and quantitative results for CoCr Ti Ha (control)

	RESPONSE INDEX	OD	MEAN OD	% OF NEGATIVE CONTROL	% OF REDUCTION
Test article (pure)	1 1 1	1.670 1.451 1.437	1.519	93.3%	6.7%
Positive control (pure)	3 3 3	0.482 0.545 0.433	0.487	29.9%	70.1%
Negative control (pure)	0 0 0	1.689 1.553 1.645	1.629	100.0%	0.0%
Test article (50%)	0 0 0	1.766 1.785 1.523	1.691	103.8%	-3.8%

Test article (25%)	0 0 0	1.603 1.479 1.567	1.550	95.1%	4.9%
Test article (5%)	0 0 0	1.695 1.571 1.590	1.619	99.4%	0.6%

Table: Qualitative and quantitative results with 20 mg gentamicin

	RESPONSE INDEX	OD	MEAN OD	% OF NEGATIVE CONTROL	% OF REDUCTION
Test article (pure)	/*	1.637			
	/*	1.731	1.645	101.0%	**-1.0%**
	/*	1.567			
Positive control (pure)	3	0.482			
	3	0.545	0.487	29.9%	70.1%
	3	0.433			
Negative control (pure)	0	1.689			
	0	1.553	1.629	100.0%	0.0%
	0	1.645			
Test article (50%)	2**	1.876			
	2**	1.823	1.787	109.7%	**-9.7%**
	2**	1.663			
Test article (25%)	0	1.695			
	0	1.590	1.647	101.1%	**-1.1%**
	0	1.657			
Test article (5%)	0	1.726			
	0	1.724	1.723	105.8%	**-5.8%**
	0	1.719			

* Particles on the cells

** Red neutral particles which interfere in the OD of these three wells, OD artificially high compared to the response index of 2

Table: Qualitative and quantitative results with 40 mg gentamicin

	RESPONSE INDEX	OD	MEAN OD	% OF NEGATIVE CONTROL	% OF REDUCTION
Test article (pure)	3	0.170			
	3	0.177	0.170	11.1%	**88.9%**
	3	0.164			
Positive control (pure)	3	0.482			
	3	0.545	0.487	31.6%	68.4%

	3	0.433			
Negative control (pure)	0 0 0	1.528 1.531 1.559	1.539	100.0%	0.0%
Test article (50%)	2 2 2	1.034 0.610 0.543	0.729	47.4%	**52.6%**
Test article (25%)	2 2 2	1.268 1.307 1.197	1.257	81.7%	**18.3%**
Test article (5%)	0 0 0	1.488 1.513 1.582	1.528	99.2%	**0.8%**

Table: Qualitative and quantitative results with 80 mg gentamicin

	RESPONSE INDEX	OD	MEAN OD	% OF NEGATIVE CONTROL	% OF REDUCTION
Test article (pure)	3 3 3	0.135 0.112 0.111	0.119	7.8%	**92.2%**
Positive control (pure)	3 3 3	0.482 0.545 0.433	0.487	31.6%	68.4%
Negative control (pure)	0 0 0	1.528 1.531 1.559	1.539	100.0%	0.0%
Test article (50%)	3 3 3	0.109 0.083 0.089	0.094	6.1%	**93.9%**
Test article (25%)	2 2 2	0.688 0.625 0.778	0.697	45.3%	**54.7%**

Test article (5%)	0 0 0	1.686 1.783 1.714	1.728	112.2%	**-12.2%**

Results and conclusions apply only to the test article tested. No further evaluation of these results is made by BIOMATECH. Any extrapolation of these data to other samples is the responsibility of the Sponsor. All procedures were conducted in conformance with NF EN ISO/CEI 17025 (September 2005) Quality Standards and the program n° 130-1 from the COFRAC (French Committee for Accreditation) « Bio cytotoxicity « in vitro » determination assays of materials and medical
devices » - Tests for in vitro cytotoxicity - code CV.10.

ADHERENCE TO PROTOCOL / STANDARD

The positive control was not conformed to Biomatech specifications. But the results were accepted because the positive control still showed a severe cytotoxicity with a response index of 3 at the qualitative evaluation.

CONCLUSION

Under the conditions of this study, the extract of the test article showed the following results for CoCr Ti Ha (control):

Concentration	Interpretation	Percentage of reduction of the cells density compared to the negative control
pure	Slightly cytotoxic	6.7 %
50 %	Not cytotoxic	-3.8 %
25 %	Not cytotoxic	4.9 %
5 %	Not cytotoxic	0.6 %

The negative and positive controls performed as anticipated.

Under the conditions of this study, the extract of the test article showed the following results with 20 mg gentamicin:

Concentration	Interpretation	Percentage of reduction of the cells density compared to the negative control
pure	No determinate because too much particles on the	-1.0 %

	cells	
50 %	Moderately cytotoxic	-9.7 %*
25 %	Not cytotoxic	-1.1 %
5 %	Not cytotoxic	-5.8 %

* No percentage of reduction of the cell density because of the red neutral particles on the cells

The negative and positive controls performed as anticipated.

Under the conditions of this study, the extract of the test article showed the following results with 40 mg gentamicin:

Concentration	Interpretation	Percentage of reduction of the cells density compared to the negative control
pure	Severely cytotoxic	88.9 %
50 %	Moderately cytotoxic	52.6 %
25 %	Moderately cytotoxic	18.3 %
5 %	Not cytotoxic	0.8 %

The negative and positive controls performed as anticipated.

Under the conditions of this study, the extract of the test article showed the following results with 80 mg gentamicin :

Concentration	Interpretation	Percentage of reduction
pure	Severely cytotoxic	92.2 %
50 %	Severely cytotoxic	93.9 %
25 %	Moderately cytotoxic	54.7 %
5 %	Not cytotoxic	-12.2 %

The negative and positive controls performed as anticipated.

DATA RECORDING AND ARCHIVING

All study file data including raw data (with blocks and slides of histological specimens), protocols, reports and ancillary documents submitted by the Sponsor, at the time the order was placed will be archived at BIOMATECH for 5 years from mailing of the final report. After the 5 years period, these items will be subject to destruction unless specific and written instruction to return them to the Sponsor is provided to BIOMATECH. Sponsor confidentiality will be protected at all times.

REPORT BY GOTHENBURG UNIVERSITY

PROJECT DENDRITES: On the optimum combination of drugs and coatings to promote early fixation and maintained long-term osteointegration

Background

Prosthetic implants are well-established treatment for osteoarthritic and trauma patients and account for over 1,4 million operations annually across Europe. However, postoperative infections are known to occur, resulting approximately 50000 cases of deep bone and 193000 cases of superficial infection. A number of implant improvements have been done in order to overcome the prosthhetic implant loosening: the improvement of bone cement, hydroxyapatite coating of the metal surface and the addition of a phospholipid-monomer as a protecting layer. However, despite advances loosening remains a problem and there are other complications of infection and inflammation that continue to cause difficulty for patients and medical services. Poor implant stability leads to the loss of either biological or cement fixation and results in wear, pain, loss of function and even fracture of the implant each of which could necessitate further surgery. The need for antibiotic therapy both orally and intravenously ranges from a period of 6 weeks to 6 months for the treatment of prosthetic infections. The appearance of antibiotic resistant bacteria and the formation of the biofilm on the implant surface demands the antibiotic concentration at the infected site 100-1000 times more than minimum inhibitory concentration (MIC). Thus, there is a need of the implant surface modification towards local drug delivery. The purpose of the study is to evaluate and characterise bone formation around candidate materials in the cortical (tibia) and cancellous (femur) bone in rabbit compared to uncoated controls used clinically. The implants and their surface modifications were prepared by the partners of the EU Dendrites consortium and delivered at the end of December 2008. The surgical implantation was performed at the beginning of January 2009. The explantation procedure has been performed, and samples are currently undergoing embedding, following by sectioning and analysis. The results will be presented in the FINAL REPORT 090801.

Materials and Methods

Implants

CoCr straight cylinders 3,75mm x 4mm with screwdriver groove in the center were coated with titanium (Ti) and hydroxyapatite (HA). Further, the implants were coated with either totally 10 mg Gentamicin encapsulated in HA slurry (coating A) or 6mg Gentamicin encapsulated in polylactide-polyglycolide (PLGA) (coating B) or left uncoated (controls).

Animals

Nine adult female New Zealand White rabbits weighing 5-6 kg, were used. The animal were studied for 6 weeks, kept in separate cages and fed a standard diet and tap water. The experiments were approved by the Local Ethics Committee, University of Gothenburg.

Surgery

The animals were anaesthetized by intramuscular (i.m.) injections of a combination of phentanyl and fluanizone (Hypnorm Vet, Janssen, Bryssels, Belgium) (1mg/kg body weight (b.wt) and intraperitoneal (i.p.) injections of diazepame (Stesolid, Dumex, Copenhagen, Denmark) (2,5mg/kg b.wt). The limbs were carefully shaved and disinfected with chlorhexidin (0.5 mg/ml) (Pharmacia AB, Stockholm, Sweden). Lidocaine (10 % Xylocain, Astra Zeneca, Mölndal, Sweden) was infiltrated subcutaneously to obtain local anaesthesia. A careful surgical technique was applied under aseptic conditions. After an incision through the skin and periosteum, a flap was raised to exposure the bone. All hard tissue preparation was performed under generous irrigation with sterile saline (NaCl 0,9%, Baxter Healthcare Corporation, Chicago, Illinois, USA) using a dental handpiece at a speed of 40.000. Each rabbit received totally 6 implants, 2 implants in each tibia and 1 implant in each femur. Three rabbits received the control implants, three rabbits - coating A and three ravvits - coating B. The implants were anchored into the bone with a screw driver. All wounds were closed in three layers: the periosteum and muscle fascia with resorbable Vicryl sutures and the skin with Monocryl sutures. Postoperatively, the animals were given an oral injection with Bactrim for 5 days, (13 mg/kg b.wt, Roche, La Roche Ltd, Basel, Schweiz). Analgetics (0,05mg/kg b.wt., Temgesic, Reckitt and Coleman, USA) were administered as single i.m. injections daily for 3 days.

Animal sacrifice

The animals were anesthetized after 6 weeks and fixed by perfusion with 2,5% glutaraldehyde in 0,05M sodium cacodylate buffer, pH 7,2. The implants and the surrounding bone tissue were removed en bloc, further immersed in glutaraldehyde for 2-3 days. After fixation the specimens were dehydrated in a graded series of ethanol, infiltrated in plastic for 1-2 weeks and finally embedded in plastic resin (LR White, The London Resin Co Ltd, Hampshire, UK).

The specimens were divided longitudinally by sawing (Exact cutting and grinding equipment, Exact Apparatebau, Norderstedt, Germany) and ground sections of 15-20 µm prepared and stained with Toluidine blue (1 % Toluidin blue and 1% Pyronin G in 1% Borax).

Light microscopy and morphometry

Light microscopic morphometry will be performed on the ground sections using an Eclipse E 600 light microscope (Nikon, Kawasaki, Kanagawa, Japan) and connected computer software. The specimens will be evaluated with respect to bone area and bone-to-implant contact.

SEM

The interface between bone and implant will be analysed with scanning electron microscopy.

Statistics

Statistical analysis will be performed using Mann-Whitney U-test for unpaired observation.

Results

Will be presented in the Final Report 090801

D10 Exploitation Plan

EXPLOITABLE KNOWLEDGE AND ITS USE

Exploitable Results

Overview Table

Exploitable Knowledge	**Exploitable Product(s) or Measure(s)**	**Sector(s) of Application**	**Timetable for Commercial Use**	**Patents or Other IPR protection**	**Owner** and Other Partners Involved
A – Micro-encapsulation	Micro-encapsulation of drug using HA and/or TCP	*Chemical, Medical, Healthcare & Pharmaceutical*	*6 to 12 months post project*	*The micro-encapsulation process has not yet been finalised and until such time as it has there can be not patent application.*	**Brace GmbH** Finsbury Development

B – Drug encapsulation	*Drug encapsulation using PLGA and liposomes*	*Chemical, Medical, Healthcare & Pharmaceutical*	*6 to 12 months post project*	*At present there are several potential method being investigated for the drug encapsulation and until a final method has been identified a patent will not be applied for* _	**Brace GmbH** Finsbury Development
C – Drug coated orthopaedic implants	*A range of orthopaedic implants that are drug coated*	*Medical & Healthcare*	*1 to 2 years post project*	*A patent application has been applied for on the coating of implants with drugs the patent application number is* 0722563.4._	**Finsbury Development** Teknimed Brace Hunt Medicoat

A: Micro-encapsulation

The micro-encapsulation process for coating drug with hydroxyapatite and/or tricalcium phosphate will make it adhere to the implant. This chemistry has the many potential uses in the chemical, medical, healthcare and pharmaceutical.

B: Drug encapsulation

The drug encapsulation using ultrasonication to synthesise PLGA or liposome coated drug. This chemistry has the many potential uses in the chemical, medical, healthcare and pharmaceutical.

C: Drug coated orthopaedic implants

The orthopaedic implant coated with drug will be the end-product of the study. The implants will be used by surgeons in the medical sector.

DISSEMINATION OF KNOWLEDGE

Overview table

Planned/actual dates	Type	Type of audience	Countries Addressed	Size of Audience	Partner responsible / involved
11/10/08 – 13/10/08	ISTA	Orthopaedic manufacturers and Surgeons	Global conference	100+	Finsbury
04/03/08 – 09/03/08	AAOS 08 - San Francisco	Orthopaedic surgeons	Global conference	100+	Finsbury
02/03/08 – 06/03/08	ORS 08 TBC - San Francisco	Researchers, students and surgeons	Global conference	100+	Finsbury
21/05/08 – 23/05/08	EFORT 08	Orthopaedic, general surgeons and researchers	Italian conference, European and rest of world	100+	Finsbury
24/04/08 – 25/04/08	EORS 08	European researcher and surgeons	Madrid Spain	100+	Finsbury
12/10/08 – 16/10/08	AOA 08 - Hobart	Orthopaedic manufacturers and surgeons	Global conference	100+	Finsbury
27/02/08 – 29/02/08	BHS 08 TBC	Orthopaedic manufacturers and surgeons	United Kingdom	50+	Finsbury
24/06/08 – 26/06/08	BORS 08 - Manchester	Orthopaedic manufacturers and surgeons	United Kingdom	50+	Finsbury
16/09/08 – 19/09/08	BOA 08 TBC	Orthopaedic manufacturers and surgeons	United Kingdom	100+	Finsbury
19/11/08 – 22/11/08	Medica 2008	General medical exhibition	Germany	100+	Finsbury

At this stage in the project, the industrial partners have identified potential arenas for the dissemination of the project results.

The table above indicates conferences and exhibitions that will be attended, a presentation of the results may not be presented at all of the events. It is most likely that dissemination at these events will be *via* networking activities.

PUBLISHABLE RESULTS

A patent application has been applied for, by the Coordinator Finsbury Development, on the coating of implants with drugs of the technology, the patent application number is 0722563.4. The preclinical research will also be published in 2009 by Gothenburg University, it is anticipated the Journals will be *J Biomed Mater Res B Appl Biomater*, *Biomaterials* and *Bone*. Recent publications in this research area by the prolific group at the Sahlgrenska Academy, Gothenburg University include;

Electron beam-melted, free-form-fabricated titanium alloy implants: Material surface characterization and early bone response in rabbits.
Thomsen P, Malmström J, Emanuelsson L, René M, Snis A.
J Biomed Mater Res B Appl Biomater. 2008 Nov 5

Hydroxylapatite growth on single-crystal rutile substrates.
Lindberg F, Heinrichs J, Ericson F, Thomsen P, Engqvist H.
Biomaterials. 2008 Aug;29(23):3317-23

Forearm bone-anchored amputation prosthesis: a case study on the osseointegration.
Palmquist A, Jarmar T, Emanuelsson L, Brånemark R, Engqvist H, Thomsen P.
Acta Orthop. 2008 Feb;79(1):78-85.

Technique for preparation and characterization in cross-section of oral titanium implant surfaces using focused ion beam and transmission electron microscopy.
Jarmar T, Palmquist A, Brånemark R, Hermansson L, Engqvist H, Thomsen P.
J Biomed Mater Res A. 2008 Dec 15;87(4):1003-9.

Characterization of the surface properties of commercially available dental implants using scanning electron microscopy, focused ion beam, and high-resolution transmission electron microscopy.
Jarmar T, Palmquist A, Brånemark R, Hermansson L, Engqvist H, Thomsen P.
Clin Implant Dent Relat Res. 2008 Mar;10(1):11-22.

Fibrous capsule formation around titanium and copper.

Suska F, Emanuelsson L, Johansson A, Tengvall P, Thomsen P.
J Biomed Mater Res A. 2008 Jun 15;85(4):888-96.

Stainless steel screws coated with bisphosphonates gave stronger fixation and more surrounding bone. histomorphometry in rats
Bone, Volume 42, Supplement 1, March 2008, Page S41
Karin Wermelin, Felicia Suska, Pentti Tengvall, Peter Thomsen, Per Aspenberg

A novel method for producing electron transparent films of interfaces between cells and biomaterials.
Engqvist H, Svahn F, Jarmar T, Detsch R, Mayr H, Thomsen P, Ziegler G.
J Mater Sci Mater Med. 2008 Jan;19(1):467-70. Epub 2007 Jul 3.

Bone response inside free-form fabricated macroporous hydroxyapatite scaffolds with and without an open microporosity.
Malmström J, Adolfsson E, Arvidsson A, Thomsen P.
Clin Implant Dent Relat Res. 2007 Jun;9(2):79-88.

Bone ingrowth in zirconia and hydroxyapatite scaffolds with identical macroporosity.
Malmström J, Adolfsson E, Emanuelsson L, Thomsen P.
J Mater Sci Mater Med. 2008 Sep;19(9):2983-92. Epub 2007 May 5.

The role of whole blood in thrombin generation in contact with various titanium surfaces.
Thor A, Rasmusson L, Wennerberg A, Thomsen P, Hirsch JM, Nilsson B, Hong J.
Biomaterials. 2007 Feb;28(6):966-74. Epub 2006 Nov 13.

The inflammatory cell influx and cytokines changes during transition from acute inflammation to fibrous repair around implanted materials.
Gretzer C, Emanuelsson L, Liljensten E, Thomsen P.
J Biomater Sci Polym Ed. 2006;17(6):669-87.

Advances in dental implant materials and tissue regeneration.
Ellingsen JE, Thomsen P, Lyngstadaas SP.
Periodontol 2000. 2006;41:136-56. Review.

A 5-year follow-up comparative analysis of the efficacy of various osseointegrated dental implant systems: a systematic review of randomized controlled clinical trials.
Esposito M, Grusovin MG, Coulthard P, Thomsen P, Worthington HV.
Int J Oral Maxillofac Implants. 2005 Jul-Aug;20(4):557-68. Review.

Monocyte viability on titanium and copper coated titanium.
Suska F, Gretzer C, Esposito M, Tengvall P, Thomsen P.
Biomaterials. 2005 Oct;26(30):5942-50.

The role of implant surface modifications, shape and material on the success of osseointegrated dental implants. A Cochrane systematic review.
Esposito M, Coulthard P, Thomsen P, Worthington HV.
Eur J Prosthodont Restor Dent. 2005 Mar;13(1):15-31. Review.

In vivo cytokine secretion and NF-kappaB activation around titanium and copper implants.
Suska F, Gretzer C, Esposito M, Emanuelsson L, Wennerberg A, Tengvall P, Thomsen P.
Biomaterials. 2005 Feb;26(5):519-27.

Long-term bone response to titanium implants coated with thin radiofrequent magnetron-sputtered hydroxyapatite in rabbits.
Mohammadi S, Esposito M, Hall J, Emanuelsson L, Krozer A, Thomsen P.
Int J Oral Maxillofac Implants. 2004 Jul-Aug;19(4):498-509.

Short-term bone response to titanium implants coated with thin radiofrequent magnetron-sputtered hydroxyapatite in rabbits.
Mohammadi S, Esposito M, Hall J, Emanuelsson L, Krozer A, Thomsen P.
Clin Implant Dent Relat Res. 2003;5(4):241-53.

Resorbable and nonresorbable hydroxyapatite granules as bone graft substitutes in rabbit cortical defects.
Liljensten E, Adolfsson E, Strid KG, Thomsen P.
Clin Implant Dent Relat Res. 2003;5(2):95-101.

Maintaining and re-establishing health around osseointegrated oral implants: a Cochrane systematic review comparing the efficacy of various treatments.
Esposito M, Worthington HV, Coulthard P, Thomsen P.
Periodontol 2000. 2003;33:204-12. Review.

Response of rat osteoblast-like cells to microstructured model surfaces in vitro.
Liao H, Andersson AS, Sutherland D, Petronis S, Kasemo B, Thomsen P.
Biomaterials. 2003 Feb;24(4):649-54.

Deliverable 11

Summary

The nature of this deliverable is under the category of 'prototype', so is given as a brief report showing the prototype components used for the *in vitro* and *in vivo* pre-clinical work.

For the prototypes; the stainless steel strips were plasma coated with titanium and then subsequently with hydroxyapatite. Theses were then coated with the encapsulated drug (either gentamicin or alendronate), when encapsulated using a polymer or coating material (PLGA-PVA, liposomes, eutragit or carbopol).

Introduction

This report shows photographs of the prototype demonstrator, as well as the synthesised microspheres of polymer encapsulating drug, the stainless steel implants with plasma sprayed hydroxyapatite.

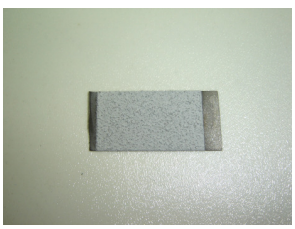
The synthesis of the encapsulated drug was conducted at Pera using liposomes or PLGA-PVA mixture. These were used to coat either alendronate or gentamicin.

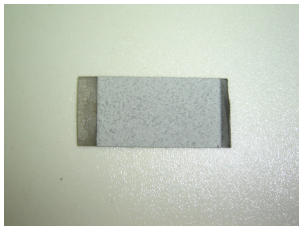
Figure 1 Photograph of a vial of the encapsulated gentamicin using PLGA-PVA

Figure 2 Photograph of a vial of the encapsulated gentamicin using liposomes

Medical grade stainless steel strips to be used for the prototype were plasma coated with titanium and then hydroxyapaptite. This work was conducted by medicoat. The grey rough surface on the implants shows the hydroxyapatite successfully ahdereing to the metal surface.

Figure 3 Stainless steel strips plasma coated with titanium and then hydroxyapatite





Subsequently, the hydroxyapaptite surface was coated with the polymer encapsulating drug. This was conducted by Pera (sonication encapsulation methodology) and Brace (film coating methodology). In total 10 different coated implants were made as prototypes which would be used for pre-clincial in vivo and in vitro testing.

Table of the coated implants made

Company	**Polymer**	**Drug**	**Process**
Pera	PLGA-PVA	Gentamicin	Sonication
Pera	PLGA-PVA	Alendronate	Sonication
Pera	Liposomes	Gentamicin	Sonication
Pera	Liposomes	Alendronate	Sonication
Brace	Carbopol	Gentamicin	Film coating
Brace	Eutragit	Gentamicin	Film coating
Brace	Eutragit	Gentamicin at pH 2	Film coating
Brace	Eutragit	Alendronate	Film coating

Figure 4 PLGA-PVA encapsulating gentamicin coated on hydroxyapatite implant

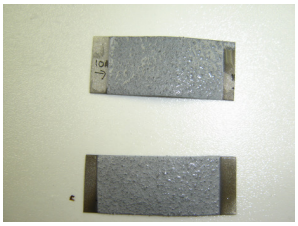


Figure 5 Liposomes encapsulating gentamicin coated on hydroxyapatite implant

Figure 6 Carbopol film coating with gentamicin coated on hydroxyapatite implant (in packaging ready to be sent for pre-clincial testing).

Figure 7 Eutragit film coating with gentamicin coated on hydroxyapatite implant (in packaging ready to be sent for pre-clincial testing).

Results and Discussion

Prototype implants have been successfully demonstrated. Stainless steel strips were plasma coated with titanium and then hydroxyapatite. These were then coated with the appropriate drug encapsulated polymer. There are 10 examples of these implants being coated with a drug encapsulating polymer. These prototype implants will be used for the subsequent step, which is the pre-clinical *in vivo* and *in vitro* work to be conducted.

Cost Benefit Analysis

Prosthesis implantation of the hip and knee are among the most common operations carried out in the EC and account for over 1.4m operations annually across Europe per year. However, postoperative infections are known to occur resulting in approximately 50000 cases of deep bone and 193000 cases of superficial infection with the effect of having to remove 7% of all prosthetic implants and in severe cases 9% of these result in limb amputation.

Our project has developed a product that adapts new technologies to meet the market place challenges from non-EU competition, through the development of a surface coating designed to deliver antibiotics and drugs aimed to promote bone growth.

We anticipate this new surface coating will:

- Reduce the number of infections within the bone and surrounding soft tissue by 60% for those fitted with the new coated implants saving 140000 patients from the risk of infection.
- Increase European implant sales by 10% and turnover by approximately €45.5 million per annum, global implant sales by 2.5% and turnover by approximately €195 million (within five to ten years of the end of the proposed project).
- Reduce overhead costs of health authorities by €116 million per annum through the reduction of post operative infections.
- Obtain 10% of the market share by 2015.

By the end of year 5 after the project, we intend, through a network of trans-national and cross-sectorial licensees, to sell the encapsulation technology for the incorporation of drugs to both promote bone growth and provide antibiotic drug delivery over a pre-defined time period.

- Generate a new market sector which will compete with the USA drug manufacturers for antibiotics, displacing at lease 2.5% of the estimated €1.5 Bn p.a Global imported implants in to Europe and allowing the group of SME's to gain a footing into the Global market place by the development of the new process.

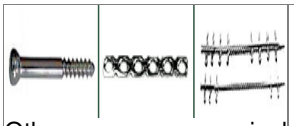
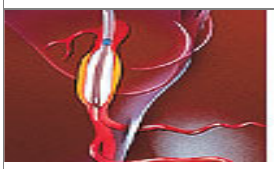
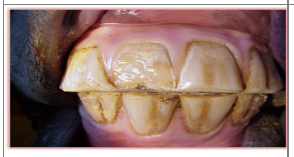
- Obtain for the SME partnership an additional 10% of the 1.4 million implant sales (140,000 additional implant sales) over the next ten years. We estimate that based on the average cost for the manufacture of Hip and Knee implants of €1,300 with an average profit of €325 we will increase the profit of the SMEs involved by €45.5 million per annum.

- The increase in turnover will have the effect of increasing employment within SMEs companies based on 1 person per €140,000 by (140,000 X 1300 = 182m / 140,000) an estimated 1300 jobs after the 10 year period.

- We also estimate that this coating will generate €116,000 million pa of healthcare cost savings due to the avoidance of complications in 140,000 patients from deep bone or soft tissues infections. However, we realise that this money will not be saved by the health care units but will actually allow them to reallocate this funding other areas of the health care sector, allowing these sectors to benefit from the additional funding.

Through the Dendrite project we has developed a technology platform, to develop a surface coating that will release drugs such as antibiotics into the site of the operation for a pre-determined time period in order to fight post-operative infections resulting in the use of less antibiotic drugs. This technology has adapted and developed beyond the limits of this proposal to benefit and strengthen large communities of SMEs across a number of different commercial sectors.

Affected Community of Companies		
Technology Application	Supply Chain Community	Distribution & End User Community
 Prosthesis Manufacturers	5 SME participants (primary & supporting supply chain) 10-15 SMEs in supplemental supply chain	20-30 Distributors/re-sellers and associated service companies 800 – 1000 SME private clinics National healthcare providers from across Europe

 Other surgical implants/objects: pins screws etc.,	5 SME participants (primary supply chain) 40-50 SMEs in the secondary and supplemental supply chain	40-50 large & small distributors/re-sellers 100 – 200 SME specialist clinics National healthcare providers from across Europe
 Dental Applications	5 SME participants (primary supply chain) 10-20 SME/LE secondary supply chain	50-100 large & small Distributors/re-sellers 10,000s of pan-European SME dental practices State funded dental clinics from across Europe 1500 – 2000 SME dental engineers.
 Stent Coating	5 SME participants (primary supply chain) 5-10 SME/LE secondary supply chain	10-15 Distributors/re-sellers and associated service companies 200 – 300 SME private clinics National healthcare providers from across Europe
 Veterinary Applications	5 SME participants (primary supply chain) 10-20 SME/LE secondary supply chain	30-80 large & small Distributors/re-sellers 10,000s veterinary and agricultural SMEs from across Europe

The table above illustrates the wide ranging commercial potential of the novel, implant-based drug delivery technology encapsulated in this proposal and the impact that the DENDRITE project has on the communities of SMEs. The focus of our project is on the integration of two key technologies, the first is the encapsulation of drugs while the second is the delivery of these drugs over a pre-determined period. Once the coating and delivery system have been proven for hip and knee prosthesis implantations then subsequent applications such as the pins, screws (used during trauma operations), dental implants, arterial and vascular stents have also be coated.

The table demonstrates that the DENDRITE project will support and benefit a large community of approximately 10,000 to 15,000 SMEs from across Europe, 5 years post-project. In all cases, the technology developed in this project has complemented and strengthened the strategic capability of the SMEs. European companies that compete against global competition on the basis of production cost and quality can adopt the DENDRITE technology (*via* pan-European partnerships and licences) and make products with new market differentiation as described in the above table. The increasing importance of cost and performance to healthcare organisations has provided added importance for this product. Our SME consortium and the subsequent post project exploitation partnership SME's (*via* license agreements) has developed and obtained unique knowledge in the coating chemistry

and coating process and the specifics of implementation. This process has reduced our vulnerability to lower cost imports from other global regions. This improved functionality and performance of prosthesis products has allowed us to exploit the knowledge and technology developed in this project on a more global scale. This has also allowed us to reduce our dependence on selling undifferentiated prostheses, mainly on price, and reduced our dependence on our domestic markets and helped us develop new internationalisation strategies for the future.

There are approximately 10 million hip and knee implants manufactured per annum globally by over 7,000 companies world wide with the majority of these company's being based in the US and India. Currently the number of implant operations is rising by approximately 15% per annum and subsequently the number of prosthesis implants being manufactured is increasing accordingly. It is estimated that if this rate of increase continues by 2010 the number of hip and knee implants being manufactured will be approximately 23 million costing over €110 billion with an average cost per implant of €4750.

Prosthesis implantations are some of the most common operations performed across Europe annually. However, complications such as soft tissue and deep bone infections are known to occur and may not be detected for days, weeks or even months following surgery. Implanted materials, such as hydroxyapatite found on the surface of joint replacements are susceptible to the formation of bacteria and unfortunately our immune system is unable to attack bacteria that live on these implants, and therefore infections become a serious problem. Large doses of antibiotics are therefore administered although the majority is absorbed by the body before they reach the site of infection. Patients with deep bone infections often require removal of the implant in order to cure the infection by either debridement or replacement of the joint with an antibiotic fixture until the infection has been eliminated. This results in the patient being incapacitated for several months while the infection is treated.

An estimated 1.4 million prosthesis operations performed per annum in the EC (based on statistical figures for the German hip and knee implantation market and using a population of 455 million for the new EC25), approximately 3.6% (50,000) of these result in deep bone infections costing the EU health authorities approximately €302 million per annum. Approximately 50,000 (7%) will require removal of the implant followed by the insertion of an antibiotic spacer while the cavity formed and now infected is cured by the administration of intravenously fed antibiotics costing the health authority in Europe in excess of €81 million per annum. Soft tissue infections account for approximately 14% (193,000) of cases of infection per annum costing in the region of €861 million per annum. So the total cost to the EU is estimated to be 243,000 citizens who will become incapacitated for an average of 3 months costing the local heath authorities an estimated €1.2 billion.

Late infections occur months or years after the joint replacement surgery, and almost always require

removal of the implant followed by the placement of an antibiotic spacer and IV antibiotics. Patients who under go this surgery require at least 6 weeks of IV antibiotics, possibly more, before a new joint replacement can be put back. Constant attention especially of elderly patients would therefore be essential with typical home care nurses earning €300 per day and being able to attend to four patients on a rotational basis. It is estimated that the cost of this additional care is currently costing the European Health authority's, based on 40% of all the patients leaving hospital needing this care for the 14 day period in the region of €408.2 m.p.a.

Conclusion

Overall, prototypes implants have been made to be used in the pre-clinical work. These prototypes are composed of medical grade stainless steel plasma coated with hydroxyapatite and then with the drug encapsulated polymer. This has been justified with a thorough cost benefit analysis.

Deliverbale 12

Introduction

The objective of the DENDRITE project was to develop a surface coating for implants such as hip and knee prosthesis that could release antibiotics (gentamycin in our application) to prevent periprosthetic infections, and also hydroxyapatite to encourage bone integration and development. During the course of the project the release of the antibiotic was assessed using various polymers such as PLGA and carbopol but also other materials such as tricalcium phosphate, hydroxyapatite and liposomes.

The proposed developments in a project of this nature do not have issues which could have an impact in relation to the delivery of the project or exploitation of the project results. Clearly, in exploiting this or any other project, it is in the interests of both the consortium and indeed the commission who are funding the project to ensure that the technology can be adopted by as wide a range of individuals as possible. This report looks into the assesses the current gender, societal and ethical issues that are currently associated with delivering the DENDRITE project and considers the potential impact on the exploitation of the project results.

In general we have been unable to achieve the objectives set for this deliverable which includes

- Presentation of two papers at three conferences and three major exhibitions
- Production of two publications in the form of editorials, technical papers and trade press.

However we believe that trying to publish our results in the public domain would have jeopardised our right to patent the technology. For this reason we have not achieved the objectives set in this deliverable.

However, we shall be looking to publish results from the outcome of the pre-clinical trials. Before we do this we will patent the technology.

The following exhibitions have been attended as part of the dissemination activities where a large had audiences attended. The table below indicates conferences and exhibitions that have been attended.

Planned/actual	Type	Type of	Countries	Size of	Partner

dates		audience	Addressed	Audience	responsible / involved
11/10/08 – 13/10/08	ISTA	Orthopaedic manufacturers and Surgeons	Global conference	100+	Finsbury
04/03/08 – 09/03/08	AAOS 08 - San Francisco	Orthopaedic surgeons	Global conference	100+	Finsbury
02/03/08 – 06/03/08	ORS 08 TBC - San Francisco	Researchers, students and surgeons	Global conference	100+	Finsbury
21/05/08 – 23/05/08	EFORT 08	Orthopaedic, general surgeons and researchers	Italian conference, European and rest of world	100+	Finsbury
24/04/08 – 25/04/08	EORS 08	European researcher and surgeons	Madrid Spain	100+	Finsbury
12/10/08 – 16/10/08	AOA 08 - Hobart	Orthopaedic manufacturers and surgeons	Global conference	100+	Finsbury
27/02/08 – 29/02/08	BHS 08 TBC	Orthopaedic manufacturers and surgeons	United Kingdom	50+	Finsbury
24/06/08 – 26/06/08	BORS 08 - Manchester	Orthopaedic manufacturers and surgeons	United Kingdom	50+	Finsbury
16/09/08 – 19/09/08	BOA 08 TBC	Orthopaedic manufacturers and surgeons	United Kingdom	100+	Finsbury
19/11/08 – 22/11/08	Medica 2008	General medical exhibition	Germany	100+	Finsbury

A report on the standards, ethical and regulatory aspects of the exploitation of the results has already been completed in deliverable 18.

Dendrite deliverable 13 – Industrial and Economic Validation

Summary

27 companies have been identified to be contacted directly to promote the project results. 27 companies will be stimulated to apply or use the results in their future strategy. 10 companies to engage in detailed knowledge or technology transfer, 3 years post project completion. 5 European companies facilitated to adopt the results in the generation of new products and services, 3 years post completion.

Relevant companies within the orthopaedic industry have been identified. At this stage they have not been contacted because the precise details of the project technology outcome remain to be finalised.

JRI Limited

8 Broadstone Place

London

W1U 7EP

Zimmer Limited

The Courtyard

Lancaster Place

South Marston Park

Swindon SN3 4FP

Telephone: 01793 584500

Stryker Orthopaedics

Cite-Centre

Grand-Rue 92, P.O. Box 1568

1820 Montreux, Switzerland

+41 21 966 12 01

J&J DePuy Orthopaedics

St Anthonys Road

Beeston

Leeds LS11 8DT

Telephone: work 0113- 270 0461

J&J DePuy Spine

St Anthonys Road

Beeston

Leeds LS11 8DT

Telephone: work 0113- 270 0461

J&J DePuy Mitek

325 Paramount Dr

Raynham, MA 02767

(001) 508 880-8100

B. Braun Melsungen AG

Carl-Braun-Straße 1

34212 Melsungen

Germany

Tel.: ++49 (0) 56 61 71-0

Biomet UK Ltd

Dorcan Industrial Estate

Murdoch Road

Swindon

Wiltshire

SN3 5HY

Tel: +44 (0) 1793 644 111

Synthes GmbH

Glutz Blotzheim-Str. 1-3

4500 Solothurn

Switzerland

Tel. +41 32 720 40 60

Corin Group PLC
The Corinium Centre
Cirencester
Gloucestershire
GL7 1YJ

Tel: +44 1285 659866

Medtronic International Trading Sàrl

Route du Molliau 31

Case Postale

CH-1131 Tolochenaz

Switzerland

Phone: +41 (41 21) 802 7000

Amedica Corporation

615 Arapeen Drive

Suite 302

Salt Lake City

Utah 84108

(001) 801.583.5100
Tornier, Inc.

11035 Roselle Street

San Diego, CA 92121

+1 (858) 866-0660 (International)

ScientX

Bâtiment Calypso - Parc Ariane 3

5, rue Alfred Kastler

78284 Guyancourt FRANCE

TEL +33 1 39 30 69 30

OsteoMed

3885 Arapaho Road

Addison, TX 75001

International: 001.972.677.4600

NovaBone Products LLC

1551 Atlantic Blvd, #105

Jacksonville, FL 32207

Tel: (001) 904-807-0140

Exactech (Uk) Ltd

Grosvenor House

Prospect Hill

Redditch

B97 4DL

+44 (0)1527 591 555

Orteq Ltd

10 Greycoat

Place,

London

SW1P 1SB

United Kingdom

+44 207 9606070

Titan Spine, LLC

Mequon Technology Center

6140 W. Executive Drive

Suite A

Mequon WI 53092

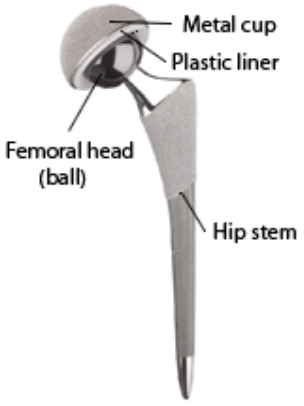
Toll Free : 1.866.822.7800

Deliverable 14

This report covers the work carried out from Month 0 – Month 28 of the project. The main body of this report is, at the request of the industrial partners, an overview. However more detailed appendices are attached to cover the work programme, and the reports on specific task deliverables. The proposed CRAFT research project, **DENDRITE**, proposes to develop a surface coating for prosthesis implants such as hip and knee implants that will release drugs for the promotion and early fixation of the implants and for the prevention of infections. This will be done by the incorporation of a surface coating that will have both free form hydroxyapatite and polymer encapsulated antibiotic drugs and bone promotion agent that will be released over a time period.

The technical work over the first 6-month period (1st September 2006 – 31st August 2007) including the month 3 and month 6 meetings have been spread over the tasks in the following Work Packages. Work Package 1 – Characterisation of Drug Morphology, Work Package 2 – Synthesis of Novel Monomer / Polymer System, Work Package 3 – Encapsulation of Drug into Novel Polymer System & Work Package 4 – Coating Trials and Method Development.

Project Management, Co-ordination, Exploitation will be on going through the life of the project. The majority of tasks in Work Package 1 are now complete and good results have been achieved. Reports on each of these tasks have been attached to this document. Work on the initial tasks within Work Packages 2, 3 and 4 have also commenced. A kick-off meeting was held at the European Commission, Brussels, Belgium, on 20th September 2006. The project goals, work plan and initial actions were all successfully presented during the meeting and agreed. The month 3 management meeting was held at Brace GmbH, Karlstein, Germany on 24th January 2007. The month 6 management meeting was held at Medicoat AG, Mägenwil, Switzerland, on 25th April 2007. There have also been several technical meetings held in between the main management meetings. During the 12 months significant progress has been made on the scheduled work package.



The successful month 15/18 meeting was held at Finsbury, UK on 25th February 2008. The month 21 meeting was held in Amsterdam on 28th May 2008. The final project meeting (month 24) was hosted as a pan-European phone conference between all the partners. The technical work over the 2nd period (16 months) (1st September 2007 – 31st December 2008) including the months 15/18, month 21 and month 24 meetings have been spread over the tasks in the following Work Packages. Work Package 5 - Integration of the Encapsulation & Coating Technology, Work Package 6 – Innovation Related Activities, Work Package 7 – Consortium Management. All work activities are running on schedule. Project Management, Co-ordination, Exploitation will be on going through the life of the project. The tasks are now complete and good results have been achieved. Reports on each of these tasks have been attached to this document. There have also been several technical meetings held in between the main management meetings. During the 16 months significant progress has been made on the scheduled work package. The progress, against the package, is briefly outlined:

Work Package 1 - Characterisation of Drug Morphology.
This work package has been largely completed. A strategy has been developed to determine the

most appropriate candidate drugs to use for this project.

Work Package 2 – Synthesis of Novel Monomer / Polymer System.
This work package has commenced. The designs and planning for the synthesis of the encapsulated drug with polymer have begun.

Work Package 3 – Encapsulation of Drug into Novel Polymer System.
This work package has commenced. The drug encapsulation has been accomplished *via* four different methods, where one will be taken forward for the final implant.

Work Package 4 – Coating Trials and Method Development.
This work package has commenced. The plasma coating of samples strips and animal model implants have begun.
Work Package 5 - Integration of the Encapsulation & Coating Technology.
This work package has been largely predominately completed. *In vitro* work has been successfully completed, *in vivo* work has commenced.

Work Package 6 – Innovation Related Activities.
This work package has been successfully completed. The production process of the drug encapsulated system has been investigated.

Work Package 7 – Consortium Management.
All consortium management tasks regarding the project have been completed.

A project web-page (http://dendrite.pera.com/) has been constructed so that all members of the consortium are up-to-date with progress in the project. It contains all relevant information for the project including; presentations, meeting minutes, meeting agendas and reports. The web-page is password protected and every member of the consortium has their own unique password to gain access into the portal. The site is regularly maintained and up-dated so it contains all the latest news and information.

A patent application has been applied for, by the Coordinator Finsbury Development, on the coating of implants with drugs of the technology, the patent application number is 0722563.4. The preclinical research will also be published in 2009 by Gothenburg University, it is anticipated the Journals will be *J Biomed Mater Res B Appl Biomater*, *Biomaterials* and *Bone*. There have been several recent publications in this research area by the prolific group at the Sahlgrenska Academy, Gothenburg University.

D16 Draft delivery of a plan for disseminating the knowledge

EXPLOITABLE KNOWLEDGE AND ITS USE

Exploitable Results

Overview Table

Exploitable Knowledge	**Exploitable Product(s) or Measure(s)**	**Sector(s) of Application**	**Timetable for Commercial Use**	**Patents or Other IPR protection**	**Owner** and Other Partners Involved
A – Micro-encapsulation	Micro-encapsulation of drug using HA and/or TCP	*Chemical, Medical, Healthcare & Pharmaceutical*	*6 to 12 months post project*	*The micro-encapsulation process has not yet been finalised and until such time as it has there can be not patent application.*	**Brace GmbH** Finsbury Development

B – Drug encapsulation	*Drug encapsulation using PLGA and liposomes*	*Chemical, Medical, Healthcare & Pharmaceutical*	*6 to 12 months post project*	*At present there are several potential method being investigated for the drug encapsulation and until a final method has been identified a patent will not be applied for* _	**Brace GmbH** Finsbury Development
C – Drug coated orthopaedic implants	*A range of orthopaedic implants that are drug coated*	*Medical & Healthcare*	*1 to 2 years post project*	*A patent application has been applied for on the coating of implants with drugs the patent application number is* 0722563.4._	**Finsbury Development** Teknimed Brace Hunt Medicoat

A: Micro-encapsulation

The micro-encapsulation process for coating drug with hydroxyapatite and/or tricalcium phosphate will make it adhere to the implant. This chemistry has the many potential uses in the chemical, medical, healthcare and pharmaceutical.

B: Drug encapsulation

The drug encapsulation using ultrasonication to synthesise PLGA or liposome coated drug. This chemistry has the many potential uses in the chemical, medical, healthcare and pharmaceutical.

C: Drug coated orthopaedic implants

The orthopaedic implant coated with drug will be the end-product of the study. The implants will be used by surgeons in the medical sector.

DISSEMINATION OF KNOWLEDGE

Overview table

Planned/actual dates	Type	Type of audience	Countries Addressed	Size of Audience	Partner responsible / involved
11/10/08 – 13/10/08	ISTA	Orthopaedic manufacturers and Surgeons	Global conference	100+	Finsbury
04/03/08 – 09/03/08	AAOS 08 - San Francisco	Orthopaedic surgeons	Global conference	100+	Finsbury
02/03/08 – 06/03/08	ORS 08 TBC - San Francisco	Researchers, students and surgeons	Global conference	100+	Finsbury
21/05/08 – 23/05/08	EFORT 08	Orthopaedic, general surgeons and researchers	Italian conference, European and rest of world	100+	Finsbury
24/04/08 – 25/04/08	EORS 08	European researcher and surgeons	Madrid Spain	100+	Finsbury
12/10/08 – 16/10/08	AOA 08 - Hobart	Orthopaedic manufacturers and surgeons	Global conference	100+	Finsbury
27/02/08 – 29/02/08	BHS 08 TBC	Orthopaedic manufacturers and surgeons	United Kingdom	50+	Finsbury
24/06/08 – 26/06/08	BORS 08 - Manchester	Orthopaedic manufacturers and surgeons	United Kingdom	50+	Finsbury
16/09/08 – 19/09/08	BOA 08 TBC	Orthopaedic manufacturers and surgeons	United Kingdom	100+	Finsbury
19/11/08 – 22/11/08	Medica 2008	General medical exhibition	Germany	100+	Finsbury

At this stage in the project, the industrial partners have identified potential arenas for the dissemination of the project results.

The table above indicates conferences and exhibitions that will be attended, a presentation of the results may not be presented at all of the events. It is most likely that dissemination at these events will be *via* networking activities.

PUBLISHABLE RESULTS

A patent application has been applied for, by the Coordinator Finsbury Development, on the coating of implants with drugs of the technology, the patent application number is 0722563.4. The preclinical research will also be published in 2009 by Gothenburg University, it is anticipated the Journals will be *J Biomed Mater Res B Appl Biomater*, *Biomaterials* and *Bone*. Recent publications in this research area by the prolific group at the Sahlgrenska Academy, Gothenburg University include;

Electron beam-melted, free-form-fabricated titanium alloy implants: Material surface characterization and early bone response in rabbits.
Thomsen P, Malmström J, Emanuelsson L, René M, Snis A.
J Biomed Mater Res B Appl Biomater. 2008 Nov 5

Hydroxylapatite growth on single-crystal rutile substrates.
Lindberg F, Heinrichs J, Ericson F, Thomsen P, Engqvist H.
Biomaterials. 2008 Aug;29(23):3317-23

Forearm bone-anchored amputation prosthesis: a case study on the osseointegration.
Palmquist A, Jarmar T, Emanuelsson L, Brånemark R, Engqvist H, Thomsen P.
Acta Orthop. 2008 Feb;79(1):78-85.

Technique for preparation and characterization in cross-section of oral titanium implant surfaces using focused ion beam and transmission electron microscopy.
Jarmar T, Palmquist A, Brånemark R, Hermansson L, Engqvist H, Thomsen P.
J Biomed Mater Res A. 2008 Dec 15;87(4):1003-9.

Characterization of the surface properties of commercially available dental implants using scanning electron microscopy, focused ion beam, and high-resolution transmission electron microscopy.
Jarmar T, Palmquist A, Brånemark R, Hermansson L, Engqvist H, Thomsen P.
Clin Implant Dent Relat Res. 2008 Mar;10(1):11-22.

Fibrous capsule formation around titanium and copper.

Suska F, Emanuelsson L, Johansson A, Tengvall P, Thomsen P.

J Biomed Mater Res A. 2008 Jun 15;85(4):888-96.

Stainless steel screws coated with bisphosphonates gave stronger fixation and more surrounding bone. histomorphometry in rats

Bone, *Volume 42, Supplement 1*, *March 2008*, *Page S41*

Karin Wermelin, Felicia Suska, Pentti Tengvall, Peter Thomsen, Per Aspenberg

A novel method for producing electron transparent films of interfaces between cells and biomaterials.

Engqvist H, Svahn F, Jarmar T, Detsch R, Mayr H, Thomsen P, Ziegler G.

J Mater Sci Mater Med. 2008 Jan;19(1):467-70. Epub 2007 Jul 3.

Bone response inside free-form fabricated macroporous hydroxyapatite scaffolds with and without an open microporosity.

Malmström J, Adolfsson E, Arvidsson A, Thomsen P.

Clin Implant Dent Relat Res. 2007 Jun;9(2):79-88.

Bone ingrowth in zirconia and hydroxyapatite scaffolds with identical macroporosity.

Malmström J, Adolfsson E, Emanuelsson L, Thomsen P.

J Mater Sci Mater Med. 2008 Sep;19(9):2983-92. Epub 2007 May 5.

The role of whole blood in thrombin generation in contact with various titanium surfaces.

Thor A, Rasmusson L, Wennerberg A, Thomsen P, Hirsch JM, Nilsson B, Hong J.

Biomaterials. 2007 Feb;28(6):966-74. Epub 2006 Nov 13.

The inflammatory cell influx and cytokines changes during transition from acute inflammation to fibrous repair around implanted materials.

Gretzer C, Emanuelsson L, Liljensten E, Thomsen P.

J Biomater Sci Polym Ed. 2006;17(6):669-87.

Advances in dental implant materials and tissue regeneration.

Ellingsen JE, Thomsen P, Lyngstadaas SP.

Periodontol 2000. 2006;41:136-56. Review.

A 5-year follow-up comparative analysis of the efficacy of various osseointegrated dental implant systems: a systematic review of randomized controlled clinical trials.

Esposito M, Grusovin MG, Coulthard P, Thomsen P, Worthington HV.

Int J Oral Maxillofac Implants. 2005 Jul-Aug;20(4):557-68. Review.

Monocyte viability on titanium and copper coated titanium.
Suska F, Gretzer C, Esposito M, Tengvall P, Thomsen P.
Biomaterials. 2005 Oct;26(30):5942-50.

The role of implant surface modifications, shape and material on the success of osseointegrated dental implants. A Cochrane systematic review.
Esposito M, Coulthard P, Thomsen P, Worthington HV.
Eur J Prosthodont Restor Dent. 2005 Mar;13(1):15-31. Review.

In vivo cytokine secretion and NF-kappaB activation around titanium and copper implants.
Suska F, Gretzer C, Esposito M, Emanuelsson L, Wennerberg A, Tengvall P, Thomsen P.
Biomaterials. 2005 Feb;26(5):519-27.

Long-term bone response to titanium implants coated with thin radiofrequent magnetron-sputtered hydroxyapatite in rabbits.
Mohammadi S, Esposito M, Hall J, Emanuelsson L, Krozer A, Thomsen P.
Int J Oral Maxillofac Implants. 2004 Jul-Aug;19(4):498-509.

Short-term bone response to titanium implants coated with thin radiofrequent magnetron-sputtered hydroxyapatite in rabbits.
Mohammadi S, Esposito M, Hall J, Emanuelsson L, Krozer A, Thomsen P.
Clin Implant Dent Relat Res. 2003;5(4):241-53.

Resorbable and nonresorbable hydroxyapatite granules as bone graft substitutes in rabbit cortical defects.
Liljensten E, Adolfsson E, Strid KG, Thomsen P.
Clin Implant Dent Relat Res. 2003;5(2):95-101.

Maintaining and re-establishing health around osseointegrated oral implants: a Cochrane systematic review comparing the efficacy of various treatments.
Esposito M, Worthington HV, Coulthard P, Thomsen P.
Periodontol 2000. 2003;33:204-12. Review.

Response of rat osteoblast-like cells to microstructured model surfaces in vitro.
Liao H, Andersson AS, Sutherland D, Petronis S, Kasemo B, Thomsen P.
Biomaterials. 2003 Feb;24(4):649-54.

Deliverable 18

Introduction

The objective of the DENDRITE project was to develop a surface coating for implants such as hip and knee prosthesis that could release antibiotics (gentamycin in our application) to prevent periprosthetic infections, and also hydroxyapatite to encourage bone integration and development. During the course of the project the release of the antibiotic was assessed using various polymers such as PLGA and carbopol but also other materials such as tricalcium phosphate, hydroxyapatite and liposomes.

The proposed developments in a project of this nature do not have issues which could have an impact in relation to the delivery of the project or exploitation of the project results. Clearly, in exploiting this or any other project, it is in the interests of both the consortium and indeed the commission who are funding the project to ensure that the technology can be adopted by as wide a range of individuals as possible. This report looks into the assesses the current gender, societal and ethical issues that are currently associated with delivering the DENDRITE project and considers the potential impact on the exploitation of the project results.

Background Information

Hip and knee implantation are amongst the most common operations carried out in the EC today. In Europe there are currently over 800 000 knee operations per year and the rate is increasing by 15% p.a. meaning that by 2015 the number of operations being performed will have reached 3.2m. The main reasons for knee replacement surgery vary from correction of a birth defect or deformity to damage caused during an injury but also osteoarthritis.

Prosthetic Implants are a well-established treatment for osteoarthritis patients, victims of accident and other trauma. Surgeons perform more than a million joint replacements each year worldwide, however, these operations are not without complication and it has been generally acknowledged that key problems remain with regard to:

- post-operative **soft tissue** infections resulting in 193 000 cases of **MRSA,** and **deep bone** infections in approximately 50 000 cases per annum.
- The need for antibiotic therapy both orally and intravenously ranging from a period of 6 weeks to 6 months for the treatment of prosthetic infections.
- Poor implant stability leading to the loss of either biological or cement fixation and therefore resulting in accelerated wear, pain, loss of function and even fracture of the implant each of which could necessitate further surgery.
- Limited prosthetic durability of the implant due to poor initial stabilisation (often caused by infection) resulting in premature loosening and poor osteo-integration.

The prosthesis implant market has a number of drivers

- The demand for prosthesis implant procedures is seeing an upward trend with an increase in the aging population and a growing number of younger patients going for joint arthroplasty procedures. Broadening patient base to include younger patients below 55 years and elderly patients above the age of 80 will increase the demand for hip replacement procedures.
- The US is calculated to have the highest increase in the over 65 population by 2025 with a 2.5% increase from 2007 to 2025. Japan is expected to see the second largest increase in the over 65 population, with an increase of 1.7%. In the five major EU markets France will have the largest growth in the elderly population equal to that of Japan. A particularly interesting characteristic of the European market in comparison to the US is the relative size of the age 65+ population. This segment is very large in Europe and as this generation continues to age clearly OA will become an increasingly lucrative prosthesis market opportunity.
- The growing elderly population is more active than their previous generation. Increased awareness of disease conditions and treatment options that provide better mobility and quality of life has resulted in more patients seeking procedures such as hip and knee arthroplasty

- The impact of the 'baby boom' generation approaching retirement age will accelerate the growth in procedures and revenues in the next 5 year period.
- Increasing problems with obesity in all age groups is also causing people to develop hip problems.

Gender Issues

In accordance with Articles 2 & 3 of the Treaty of Amsterdam (1997) and other EU policy directives (COM (96) 67 final) and reports (EUR 20022) the DENDRITE partnership has throughout the project committed to incorporating the principles of gender mainstreaming throughout the various elements of the programme.[1] [2]To this end, every effort has been made to ensure that the work programmes and related activities contribute to the promotion of gender equality wherever possible, and steps have been taken to ensure that none of the activities within the programme contributes to gender inequality or aggravates existing gender inequality. The following objectives underpin the gender action plan for the DENDRITE project:

- Women and men have equal opportunities to participate in the various parts of the programme
- In addressing diversity, the work programmes took account of the different situations needs and interests of women and men.
- The work programmes in the project to contribute to reducing inequalities between women and men.

Ensuring women and men have equal opportunities to participate in the programme

With regard to promoting the active participation of women scientists in the programme:

- During the course of the project active measures were taken to ensure that women scientists were well represented in each of the partner's organisations and that women scientist were given first option to lead certain aspects of the project.
- Steering Committees and Advisory Panels were established with a minimum of 40% representation from the female gender to support individual work programmes.

[1] Communication from the Commission (1996) Incorporating equal opportunities for women and men into all community policies and activities. COM (96) 67 final

[2] European Commission 2001 Gender in Research: Gender Impact Assessment of the specific programmes of the Fifth Framework Programmes. An overview EUR 20022

- Opportunities for mobility within the programme were taken into account of the different needs of women and men in order to enhance participation by women scientists.

The DENDRITE project is gender sensitive in a number of ways, namely:

- The composition of the research personnel in the consortium is 29% which is significantly higher than the 5% female representation seen in FP5 CRAFT projects[3].

- During both the market research and product testing phase the project work plan differentiates the expected system end users on both ethical and gender grounds. This ensures that gender issues are addressed at the critical points in the product development life cycle.

Project-specific gender issues – In preparing the DENDRITE proposal and during the course of the project the Consortium performed a review of the anticipated research and development. This review concluded that there was little likelihood of any gender-specific responses influencing the development of the coated prosthetic implant or its implementation in our clinical trial. At the present time we have encountered no gender specific issues raised by the project partners to date. The partners will remain vigilant of any gender specific issues post project.

We currently do not anticipate that there will be any gender issues associated with the technology which we will exploit from the project. In addition, we can see no other potential gender issues with our other proposed areas of exploitation. However, as these areas have only been outlined during the course of the project we will continue to review any possible issues arising after the project as our exploitation plans develop.

Societal Issues

In preparing the DENDRITE proposal, the Consortium has already given a significant level of consideration to the potential societal impacts of the project. Indeed, by being funded, the project has clearly demonstrated that it has a potential impact.

Societal Issues in Exploitation

Following review of our anticipated exploitation at the time of preparing the DENDRITE proposal and a reassessment of our plan to exploit the project results we still remain

[3] Gender in Research – EC Synthesis report, EUR20022

confident that our initial analysis and predictions of societal impact will remain valid. Indeed, given the success of our project to date, we have gained additional confidence that the project will achieve its anticipated aims and deliver the impacts discussed.

In summary, we have reviewed the DENDRITE project results to date and our current exploitation plans and we can only identify positive societal benefits of exploiting our system, with no societal barriers to the exploitation of our project.

The primary application of the DENDRITE technology was to actually develop an innovative coating that has the potential to reduce post operative infections. This was to be achieved by the timely delivery of antibiotics and bone promotion drugs which we believe has the potential to reduce hospital stays and allow patients to be released early from the early. More over we know that DENDRITE technology can and will be manipulated in the trauma market. It is in this market that the coating technology would provide an indispensable defence against early infections.

It was important for us to develop the DENDRITE technology as it had the potential to allow us to coat dental implants. During the course of the project we have shown that it is possible to coat implant material with a drug loaded polymer. We held this belief before and during the course of the project. We made every endeavour to reach this as one of our objectives and in doing so it has opened up the possibility of us exploiting the technology further in the dental implant market. The coating of dental implants was seen as only a secondary application however we now believe that we can move forward into the market as well.

The impact of the project was to develop an innovative drug delivery system to deliver gentamycin. This goal was achieved early on in the project. It was vital that we could achieve this in order to build up our confidence and plan for the rest of the project. The medium term impact for the SMEs will be felt over the next 5 years by which time we will have completed any further development work but also any further pre-clinical and clinical studies in order to take the product to market. The DENDRITE partners believe that this will be possible as Finsbury have the capability of achieving this and have partners behind them to achieve further milestones.

Economic Justification for The Proposed Research

Market Size

During the course of the project we revisited the market size for the DENDRITE project. In Europe there are approximately 1.4 million prosthesis operation performed costing an estimated €13.5 billion of which some 50,000 result in deep bone infections requiring either the removal of the implant or debridement of the surgical area. In each case antibiotic drugs are administered for several weeks or months. While soft tissue infections account for 193,000 patient that have under gone prosthesis implantation costing in the region of €861.5 million per annum in additional care. The cost for remedial surgery for the removal and insertion of a new hip prosthesis following deep bone infection is estimated at €302.5 million per annum across Europe. The corresponding North American markets for hip implants is estimated to be a further €15 billion per annum. The total global market just for replacement joints is value in the region of €50 billion per annum.

The intention of the DENDRITE project is to supply the coated prosthesis implants (hip, knee, shoulder, etc.) that will reduce the possibility of short and long term post operative infections by the timed release of antibiotics. The DENDRITE project will also deliver bone promotion drugs to stimulate osteoblasts for the early fixation of the implants. Our realistic aim, by 2015 is to increase our global market share by 2.5%, which will equate to approximately €1.2 billion per annum.

Summary of the estimated cost to the European Union per annum due to infection follow joint replacement

Total number of replacement Joints operations in Germany	250,000 [4]
Total population of Europe (EU25)	455 million[4]
Population of Germany	82,4 million[20]
Average cost of joint replacement including hospital stay	€9,800
Average cost of revisional surgery for joint replacement	€15,000
Average cost for extended stay in hospital per person (number of extra days X cost of hospital bed per day)	€4,500
% deep bone infections resulting from joint replacement	3.6%
% deep bone infections resulting in replacement of the implant	7%

[4] Religious Consultation on Population, reproduction health & ethics

Average % of Superficial infection as a result of joint replacement.	14%
Calculated number of joint replacements in Europe per annum	1,4
Calculated number or revisional joint replacements in Europe per annum due to deep bone infection	3,500
Total number of people affected by deep bone infections per annum	50,000
Total number of cases of superficial infection in Europe per annum	193,000
Average cost of primary joint replacement inclusive of hospital stay	€9,800
Average cost of superficial infections needing additional treatment for 3 extra days in hospital.	€861.5 million
Average cost of additional stay in hospital due to deep bone infection	€221.5 million
Average cost of revisional joint replacement due to deep bone infection inclusive of hospital stay	€23,000
Total cost of revisional surgery due to deep bone infection	€80.9 million
Total cost of deep bone and soft tissue infection to the European health service per annum	€1.2 billion
Total estimated cost of joint replacement operation per annum	€13.5 billion

Summary of market sizes[5]

Based on the calculation in the previous table and the current market trend for implants which estimates that the prosthesis marking is currently increasing by approximately 15% per annum we have come to the following conclusion:-

- We estimate that the increase of the European market by 15 % per annum will mean that by 2015 our manufacturing of current products will have risen from 100,000 to 400,000 prosthesis implants per annum. As a result of this we estimate that we will also generate a European market share for the new coated implants of approximately 140,000 additional units over the same time period.
- This increase in new coated products will have the affect of increasing our turnover by approximately €630 million per annum.
- It is therefore estimated that as a result of the increasing market and the contribution of the additional unites being manufactured with the new coating that we the SME's involved in this project will increase our combined profit by €157.5 million during the same time period.
- The average price for the current device is approximately €1200 per set whereas we estimate that the new coating process will add another €100 to the cost of this manufacture (€1300 per unit). We therefore have used this figure combined with the predicted number of units to be sold for the calculations in the table below. We estimate that this addional cost will be absorbed by the health care systems as they will reduce the number of post operative infections by approximately 10% saving them €116 million per annum.

[5] Arthritis Research Campaign 2003

- While the direct cost of the new coating media may increase the cost of purchase to the heath authorities by €14,000 per annum they will reduce their overhead cost for treatment of patients suffering from either soft tissue or deep bone infections by €116 million per annum.
- With an estimated global market of €50 billion per annum we would look to increase our presence within this market sector by taking a 2.5% share of this over the same ten year time period giving us an estimated €1.2 turnover with a predicted €315 million profit.

Year	% Market Penetration Europe	Revenue in European market €	
		Addition sales €	Profit €
1	CE / MDA Approval	N/A	N/A
2	0.5	9 million	2.2 million
3	1.0	18.2 million	4.5 million
4	1.5	27.3 million	6.8 million
5	2.0	36.4 million	9.1 million
6	2.5	45.5 miilion	11.4 million
7	3.5	63.7 million	15.9 million
8	5.5	100 million	25 million
9	8	145.6 million	36.4 million
10	10	185 million	46.2 million
Total increase in revenue and profit as a direct result of the project		630.7 Million	157.5 Million

Predicted cost for the manufacture an additional 140,000 prosthesis units per annum which will be coated with the DENDRITE coating. This table include the sales cost including manufacturing and the profit for all the SME's.

Alternate market sectors

We have also looked at alternate market sectors and can conclude that there is a large market sector for this technology on other areas. We have specifically estimated that:-

- The trauma market and other medical device market is worth €120 per annum taking into account that part of this market will be for prosthesis implants we estimate the global trauma (pin, screws, plates, etc.) market to be €70 per annum.
- We therefore anticipate a market penetration of approximately 3.5% (€2.4 billion) could be achieved after a five to ten year period. It is intended that this alternative market would be licenses out to other SME communities within the European Union at a rate of 5%, which would give the SME's involved in this project a net income of approximately €120 million per annum.

- As for the other alternate market sectors such as the Dental industry we intend to look into the licensing of this technology into those market sectors following completion of the project.

Impact of working at a European level

Many of the partners have previously worked together on projects which involved the development of prosthetic implants with Finsbury. It was based on this decision that these partners would come together again with new partners in order to develop the DENDRITE project. However in order to this a pan-European partnership was needed in order to develop this product as not all expertise is found in any one country. Moreover we were specifically looking for partners who had the relevant skills and equipment to deliver the project but also guarantee delivery after the project. In order to do this we had finalised the partnership 6 months prior to submission so that we could all get together and evaluate each others expertise and how we could complement each other.

Through the make-up of our consortium, we provided a viable and realistic supply chain for the development of our technology, acting as a springboard from which subsequent exploitation of initially an EU wide market, and subsequently, a global market might occur.

Social cohesion

Before submission of our proposal a detailed investigation was carried out internally by our team at Finsbury to ensure that our concept would overcome many of the economic and social problems. The advances that we have made so far will not only help to expand the current implant market but will also increase the awareness of the encapsulation technology allowing this to develop into other market sectors. This will have the effect of increasing employment across a wide sector of the EU. The scope of the employment potential is particularly strong due to the annual increase (approximately 15% increase per annum) of prosthesis implantation.

The development of the drug encapsulation system will give prosthesis manufacturers across the EU a unique opportunity to increase their competitiveness against India, USA and other non-EU low labour cost regions. We also believe that not only the more prosperous member states will benefit from this technology but because the technology will not be commercially expensive to produce will give some of the poorer member states and relevant entrant countries the opportunity to compete in the global market as equals.

Economic cohesion

The adoption of this technology by the medical and chemical sectors within the poorer member states in the EU and accession states will offer them a unique opportunity to begin to compete and differentiate on more than just labour costs. It is hoped that the technology developed during the past 2 years will help the less economically developed member states and accession states to improve their products offering them a way of equalling the level of performance currently obtained by the more prosperous EU member states, resulting in an improved economic cohesion across the EU.

EC Legislation

During the course of this project every effort has been made to conform to the following ethical rules.

- The Charter of Fundamental Rights of the EU. This project has not effected any European citizens rights, it should be noted that every endeavour has been made to conform to all regulations and directive issued by the European Government
- Directive 2001/20/EC of the European Parliament and of the council of 4th April 2001 on the approximation of the laws, regulations and administrative provisions of the Member States relating to the implementation of good clinical practice in the conduct of clinical trials on medicinal products for human use. It should be noted at this point that no human trials were conducted during the course of the project and therefore this directive was not directly applicable to.
- Directive 95/46/EC of the European Parliament and of the Council of 24 October 1995 on the protection of individuals with regard to the processing of personal data and on the free movement of such data. There was no collection of personal data during the course of this project and as such no personal data was stored on any device in any format.

Conclusion

We have carried out an in-depth study on gender, societal and ethical issues. While some gender issues have been reported there were no societal issues. Therefore we have only reported on the societal impact of the project. We have also looked at EC legislation and identified some issues. This report clearly shows that there are limited barriers to the exploitation of the results