Project Number: COOP.CT-2005-018060

Project Acronym: SilkBone

Project Title: Development and Evaluation of Mineralised, Silk-Based Composites for Orthopaedic Applications

Project Instrument: Cooperative Research

Thematic Priority: Specific Research Projects for Small/Medium Sized Enterprises:

Final Activity Report

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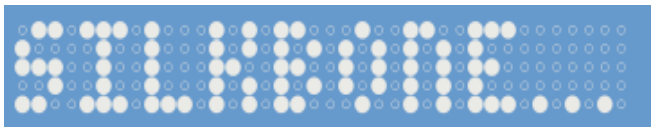
Project Logo:



Period Covered: 1st October 2006 to 31st March 2008

Date of Preparation: 18th of August 2008

Start Date of Project: 1st October 2005

Project Duration: 30 Months

Project Coordinator: Dr. Nicholas Skaer

Coordinators Organisation: Oxford Biomaterials Ltd.

{Draft 2}

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Project Execution

1. The SilkBone Consortium:

SilkBone is an E.U. sponsored Framework Programme 6 Co-operative Research project comprising an 8 partner consortium developing a novel silk-based biomaterial for bone repair. SilkBone is coordinated by Oxford Biomaterials Ltd. with the Universities of Oxford, Bristol, Southampton, (U.K.), and Konstanz (Germany), Progentix B.V. (Netherlands), 3H Biomedical A.B., and Sveriges Lantsbrukuniversitat (Sweden) being the other partners. Coordinator contact details and website URL, can be found at the foot of this document.

2. Original Project Objectives:

1. To develop a novel, mineralized, silk-based biomaterial with mechanical and biochemical features that make it suited to application in bone graft replacements.
2. To combine this generic material in a novel osteoinductive (i.e. capable of inducing bone formation), regenerated silk-based matrix to create an entirely innovative bone scaffold material. The matrix will be designed to reinforce the mechanical properties of the material created in objective 1, and encourage nucleation of mineralisation and ingress of endogenous cells through incorporation of osteoinductive factors and proprietary microporous design. The resulting scaffold will constitute the first osteoinductive, load bearing bone substitute material (BSM).
3. To develop know-how in non-foetal, mesenchymal stem cell technology for incorporation of tissue engineered cells in the bone scaffold.

3. Key Issues Addressed:

Novel implantable biomaterials and their combination with stem cells and with recombinant factors that promote cell differentiation and healing have been recognized by the UK Foresight Programme and European Commission Initiatives to be extremely important in health care technologies for the future. The clinical need in Europe for novel bone graft replacement materials is clear and has been called for by health practitioners and academics alike. The ultimate objective of the SilkBone project has been to produce an entirely novel BSM based on silk technology

developed the Project Coordinator, Partner Oxford Biomaterials, and to seed this BSM scaffold with tissue engineered non-foetal stem cells, generating a cellular, bone substitute material. The goal was to combine the following unique features in the BSM: 1. Full bio-absorption; 2. Sufficient tensile/mechanical properties to act as a stand-alone, load bearing bone graft replacement; 3. Osteoconductivity and osteoinductivity (guides and encourages bone tissue regeneration) 4. Capable of being seeded with a patient's own mesenchymal (bone marrow) stem cells.

4. Technical Approach:

Project Coordinators, Oxford Biomaterials have developed a novel silk based biomaterial platform technology which they have trademarked Spidrex®. The aim at the start of the project was to develop a composite BSM based on a mineralised silk fibre lay combined with a gelled matrix of silk proteins. In the event, such has been the progress made on the project with development of silk protein matrices and incorporation into these of the natural constituent of human bone, hydroxy-apatite mineral, that the use of a mineralised silk fibre component was rendered obsolete. The resulting silk fibroin-hydroxyapatite nanocomposites created have formed the basis of the projects success. They have been demonstrated to be biomimetic of the structure of human bone: a long chain fibrous structural protein (collagen in the case of human bone, silk fibroin in the case of the SilkBone composites) interleaved with nanocrystals of hydroxyapatite mineral. Non-foetal stem cell technology has also successfully been developed and used to seed the BSM, and we have tested human cell reactions to our BSM *in vitro*, in line with Directive 86/609/EEC.

5. Expected Impact:

A cellularised, load bearing, resorbable BSM is unprecedented (Rose and Oreffo) and will provide entry to a market estimated at €2.5 billion. It will have particular application in impaction grafting – a technique used in the increasingly common joint replacement and revision procedures (arthroplasties) where currently either the patients own bone (autograft) or bone from bone banks (allograft) is the only current option and both of which have significant associated disadvantages.

The 'SilkBone™' BSM will also be particularly advantageous for the treatment of fractures resulting from osteoporosis and bone lesions in cancer patients. We believe it will alleviate suffering and incapacity in millions of patients and lead directly to great savings in time and cost for Health Services. It is also noted that tissue engineering, adult stem cell research and implantable biomaterials have been flagged as future growth markets, keenly pursued in the U.S. and Asia. Having developed and disseminated know-how in these areas the SilkBone Consortium has also increased E.U. competitiveness in these burgeoning sectors.

6. Summary of Project Results:

The project made significant progress from the outset: at an early stage advances were made with creating high quality solutions of silk fibroin molecules and gelling these into porous silk scaffolds. This was a prerequisite for achieving a silk based BSM, and the goal was to take commercially sourced silk fibres, strip them of contaminating cytotoxic surface components and dissolve the remaining structural protein, fibroin, to provide a solution of molecules from which 3-D porous scaffolds could then be cast. However it was recognised by Partner Oxford Biomaterials early in the project that maintaining fibroin chain length was of key importance in deriving porous scaffolds with high moduli (i.e. good resistance to compressive forces). Proprietary processes were developed for processing silk fibres to ensure the highest quality silk fibroin molecules were obtained in the fibroin solutions and these processes were patented. A series of evaluations of the silk protein solutions were conducted to assess the quality of the fibroin molecules and the degree to which they had been degraded, which included microscopic observation using Nomarski optics (Figure 0) which

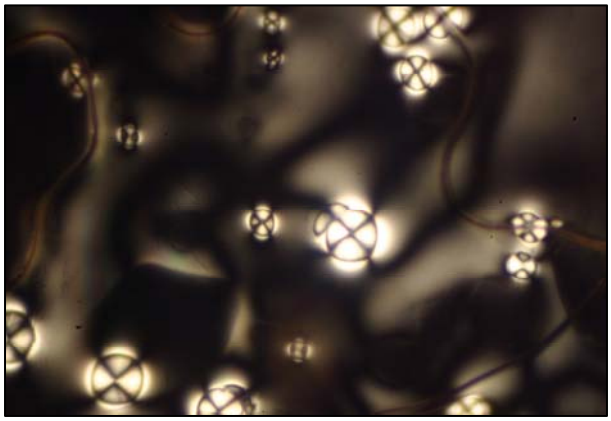

Figure 0: Optimised silk fibroin solutions viewed with Nomarski optics reveal self assembly into liquid crystalline spherulites.

revealed liquid crystalline behaviour and complex rheological analyses performed by Partner Oxford University. These confirmed that Oxford Biomaterials proprietary silk fibroin processing resulted in a solution of silk fibroin molecules with a quality that approached those found in the silk gland of silkworms prior to the fibre being spun. The optimised silk fibroin solutions were cast into 3-D porous scaffolds by modulating the temperature and pH of the solution resulting in fibroin 'sponges' with extremely interesting properties. Highly resilient and with large elastic modulus they offered a high degree of resistance to compressive forces. This led Partner Oxford Biomaterials to consider them as potential tissue regenerative scaffolds for cartilage repair, and this possibility was explored further on the project resulting in a business plan being prepared for a commercialisation vehicle called Orthox Ltd being formed to take this technology forward (see appendix 1).

Initally, efforts were made to mineralise these silk fibroin sponges directly, using hydroxy-apatite, the mineral constituent of human bone. However, this yielded poor results as mineral adhered solely to the surface of the scaffolds, resulting mechanical strengths achieved were low, and the mineral masked the silk proteins from acting as a substrate for cells to interact with. The breakthrough came in developing a method for gelation of the silk fibroin solutions using phosphate: a precursor of hydroxyapatite which subsequently allowed hydroxyapatite nano-crystal nucleation through-out the silk fibroin scaffold. This yielded first generation SilkBone™ BSM (Figure 1): A porous protein/mineral composite which testing demonstrated was capable of resisting up to four times the compressive forces that the leading competitive mineral based BSMs on the market were able to withstand.

Figure 1: Spidrex® SilkBone™ is a load bearing bone substitute material made from a nano-composite of silk protein and hydroxyapatite which remodels as bone when implanted.

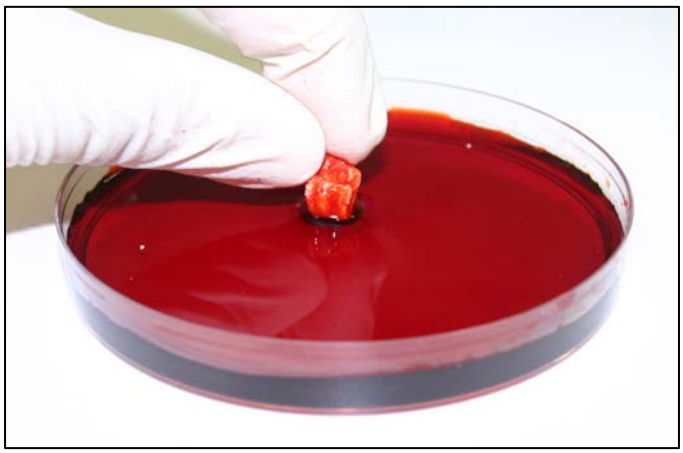

Figure 2: Spidrex® SilkBone™ being dipped into simulated blood which rapidly wicks through the porous structure. The sample remained load bearing when tested whilst retaining the fluid.

These mechanical properties clearly marked our SilkBone™ BSM out as leading the state of the art by a considerable distance. However, without equally impressive biological responses the results were meaningless. Early testing of the SilkBone BSM in cell culture and through profiling the predicted response of the human immune system to implantation of the material through measuring the release of immunomodulatory cytokines and proinflammatory factors in human whole blood assays revealed the SilkBone™ BSM would likely be entirely biocompatible and certainly permissive of bone cell and tissue growth and differentiation. Equally importantly, the SilkBone™ BSM was observed to 'wick' blood and cells into its porous structure, essential for encouraging ingress of regenerating bone tissue when implanted (Figure 2).

However, it was observed that when this occurred, the SilkBone™ BSM lost much of its key mechanical rigidity, swelling and becoming comparatively soft. It transpired that the silk fibroin matrix was absorbing water, reverting to a hydrogel state and swelling with concomitant loss of mechanical rigidity. Before embarking on the key trials of the material in the *in vivo* work package on the project, this problem required resolution. During the second part of the SilkBone project much of the efforts of two partners, Oxford Biomaterials and Bristol University were dedicated to resolving this issue and delivering a final iteration SilkBone™ BSM which would maintain its unique mechanical advantages when implanted in the body. Although a considerable project delay resulted, this was eventually achieved using a proprietary silk fibroin cross-linking protocol which was subsequently patented.

The result was not only a second generation SilkBone™ BSM capable of bearing loads comparable to human bones when wet as well as when dry, and thus meeting the targets specified at the outset of the project, but further, a material which significantly surpassed the toughness of many types of human bony tissue. Mechanical testing of the cross-linked SilkBone™ BSM revealed a ductile, non-catastrophic, failure mode which allowed the material absorb and transmit very great quantities of energy without ultimate failure of the structure, probably through a mechanism of molecular realignment. This is in marked contrast to mineral based BSM's (figure 3) which were observed to exhibit brittle behaviour and catastrophic failure at much lower compressive loads.

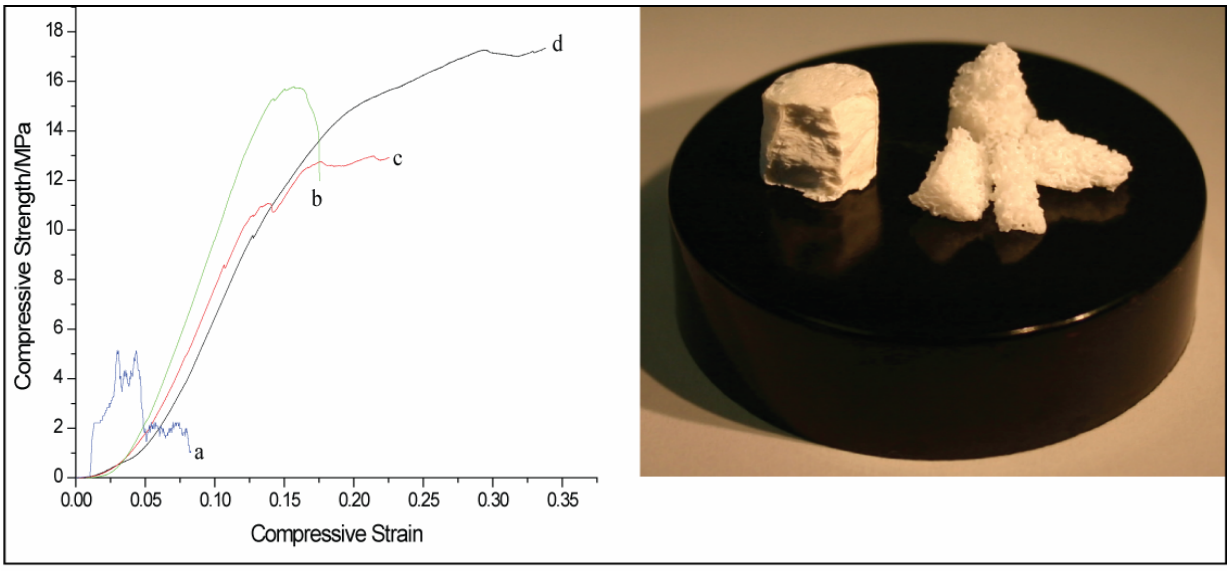

Figure 3. Left: Plots of compressive stress (%) against strain (MegaPascals - MPa) for (a) a commercial mineral-based Bone Substitute Material (BSM), and (b-d) SilkBone BSM show the increased compressive strength and extended compressive overload region for the SilkBone composite. Right: A photograph of samples following compressive loading demonstrating the catastrophic failure of the brittle, pure mineral BSM (right) and the non-catastrophic ductile deformation of the tough, resilient, silk + mineral SilkBone BSM (left).

Having overcome the final material challenges to achieving the specifications we aimed for in our SilkBone™ BSM, the second generation SilkBone™ BSM was extensively evaluated *in vitro* using the same methods as the first generation material. In addition, a novel Cell Based Biocompatibility Assay developed by Consortium Partner 3H Biomedical AB, was used to assess the final iteration BSM. They hope to commercialise this shortly for trialling other implantable biomaterials.

The second generation material demonstrated very positive results in all these preliminary biological evaluations, showing no signs of significant pyrogenic (fever inducing) contamination despite not being manufactured in clean room facilities, excellent capacity to support cell growth in culture and did not trigger immuno-modulatory cytokines suggesting it would be well tolerated by the human immune system. In recognition that the delay in proceeding to the *in vivo* evaluation of the second generation material had compromised the length of the trials that could be conducted on the project, a series of *in vitro* analyses of material's resorption rate were instigated by Partner Bristol University. These demonstrated a profound effect of the cross linking protocol in retarding the resorption rate. While the full implications will not be understood until long term *in vivo* testing is performed it is considered that the potential to tune the material's resorption rate could well open up a variety of different clinical applications in slow to heal indications for example.

Consortium Partner Progentix had, by this point, advanced their stem cell bioreactor technology to the point where large quantities of human bone marrow derived adult stem cells could be harvested and multiplied according to the schematic in Figure 4.

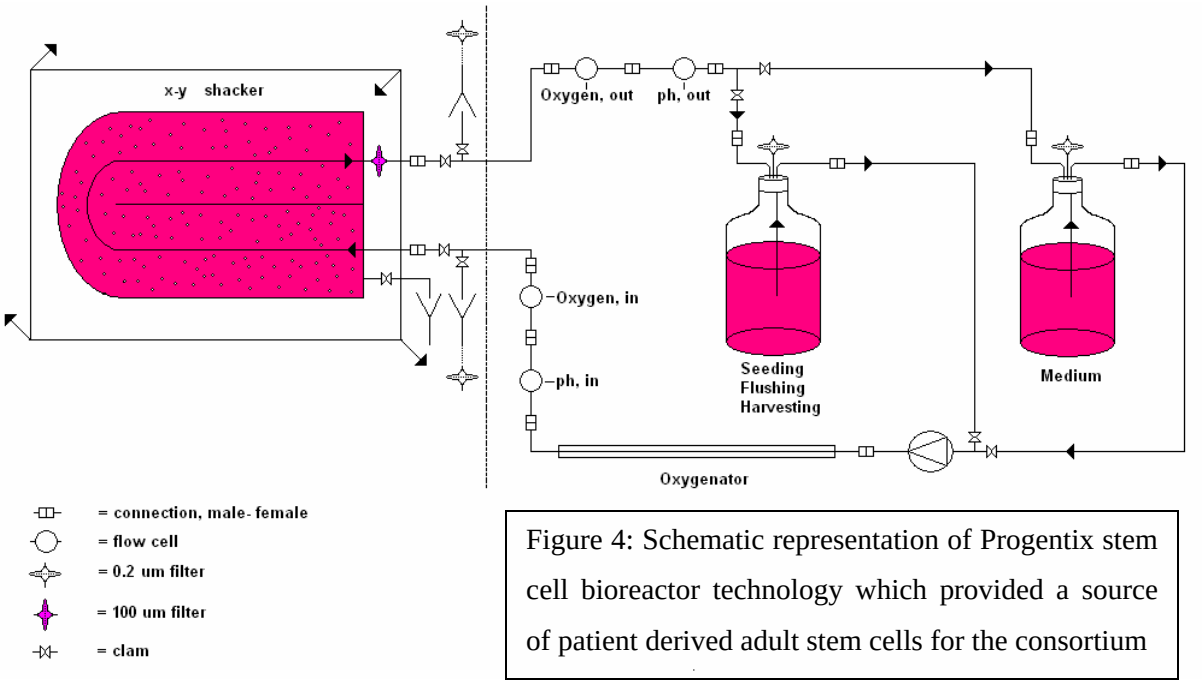

Figure 4: Schematic representation of Progentix stem cell bioreactor technology which provided a source of patient derived adult stem cells for the consortium

This permitted testing of the second generation SilkBone™ BSM with these patient derived stem cells. Despite technical difficulties inherent in working with a novel material the experiments demonstrated that patient derived cells could be seeded on the SilkBone™ BSM and proliferated well, covering the scaffold and infiltrating the porous structure. This delivered a major milestone, revealing the SilkBone™ BSM to be not only load bearing and resorbable but also capable of being cellularised.

The demonstration of biocompatibility through extensive *in vitro* evaluation of the second generation SilkBone™ BSM permitted progression of the *in vivo* work package. This was undertaken by Partner Southampton and, using a subcontract from Partner SLU to an FDA accredited private facility, Visionar AB. The material was implanted subcutaneously in rodents with no undue immune responses or inflammation observed, and all trial subjects tolerated the material without sign of either distress or irritation. The experiments demonstrated that bone cells colonise the SilkBone™ BSM, deposit newly synthesised bone tissue and begin the action of remodelling the implant and bone through the activity of multinucleate osteoclast cells (Figure 5).

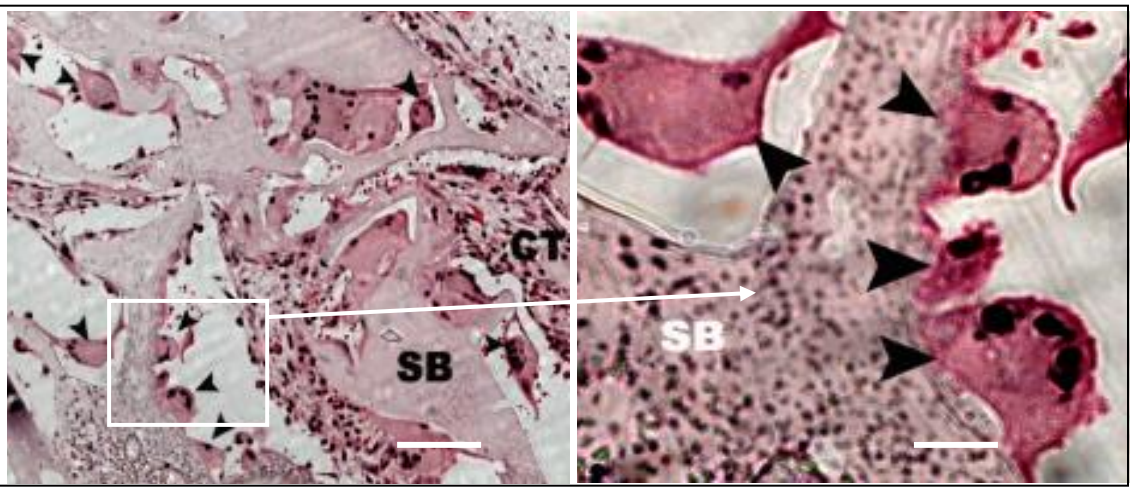

Figure 5: (left) osteoclastic remodelling (arrows) of the silk-hydroxyapatite scaffold and newly formed bone associated with the macroporous implant, scale bar =100µm; (right) magnification of the boxed area showing osteoclastic infiltration and remodelling of the newly synthesised bone through the activity of multinucleate osteoclasts (arrows), scale bar =20µm.

Further experiments also revealed strong indications that new bone tissue is being deposited as the SilkBone™ BSM is resorbed, and provided evidence that endogenous cells are recruited to the scaffold and undergo osteogenic differentiation indicating SilkBone's capacity to induce bone regeneration (Figure 6)

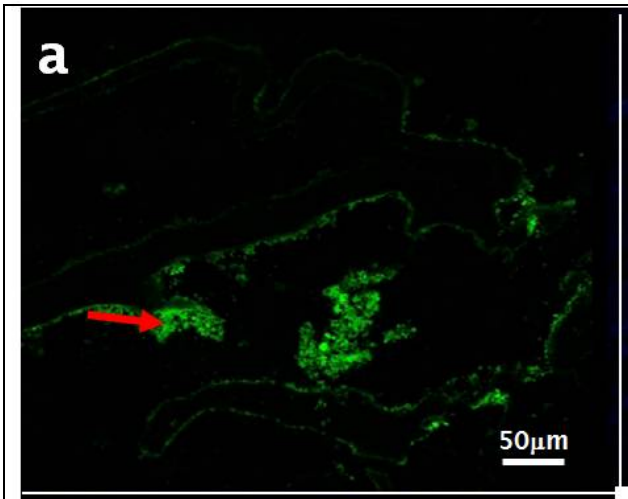

Figure 6: Confocal images of fluorescently labelled cells seeded onto Silkbone after implantation for 4 weeks [a] green labelled human cells adhere to the Silkbone surface and proliferate. [b] blue nuclear stain [c] composite image of [a] and [b], showing that murine cells with an osteoblastic morphology, have been recruited to the implant suggesting that the human cells expressed osteogenic proteins *in vivo*.

This suggests that SilkBone™ BSM, with its biomimetic bone structure, is very well suited to promoting regenerative bone healing. These observations, combined with the unique mechanical advantages offered by the material and strong patent position adopted by the consortium, mean the SilkBone project has not only delivered all the major objectives under budget and on schedule, but has also devised, developed and delivered an entirely novel biomaterial platform technology with exceptional potential for application in regenerative orthopaedic tissue repair.

7. Achievement of Major Strategic Project Objectives,

Page 4 of the SilkBone Description of Work, set out at the beginning of the project, describes three Major Strategic Project Objectives. These were to:

1. Generate a novel, mineralized, silk-based biomaterial with mechanical and bio-chemical characteristics that make it suited to bone graft replacement applications.

2. Combine this generic material in a novel osteoinductive, regenerated silk-based matrix to create an entirely innovative bone scaffold material. The matrix will be designed to reinforce the mechanical properties of the generic material created in strategic objective 1, and encourage nucleation of mineralisation and ingress of endogenous cells through incorporation of osteoinductive factors and proprietary microporous design. The resulting scaffold will constitute the first osteoinductive, load bearing, bone graft material.

3. Develop know-how in non-foetal, mesenchymal stem cell technology for incorporation of tissue engineered cells in the bone scaffold generated in Strategic Objective 2.

Strategic Objective 1

This was fully and successfully completed. Data presented in section 6 of this report and both periodic activity reports 1 and 2 clearly show that in SIlkBone™ BSM the consortium has achieved, from concept, an entirely novel silk based biomaterial with the molecular characteristics and load bearing mechanical properties to suit it extremely well to application in bone graft replacements. On WP's 1, 2, 3 and 4 hydroxyapatite/silk fibroin composites were generated using both silk fibres and regenerated silk solutions, the latter proving ideal for bone scaffolding applications. Mechanical tolerances were demonstrated on WP5 and suitability for bone grafting

procedures established on WP's 6, 7, 8 and 9. The only caveat to the data obtained was failure of a stringent biocompatibility test on WP8 which it is thought is due to non-optimised manufacturing processes currently used. It is expected this will be remedied once GLP and GMP manufacturing is established.

Strategic Objective 2

This was also completed successfully, albeit not in the manner initially envisaged in the Description of Work owing to the rapidity and high quality with which the silk matrix component on Work Package (WP) 2 was completed, and the subsequent success in directly mineralizing the silk matrix with hydroxyapatite on WP4 to achieve the final iteration SilkBone™ Bone Scaffold described in Objective 2. This mineralized silk matrix proved to have sufficient mechanical strength and osteoconductive potential in itself to meet project criteria allowing the consortium to abandon the use of a mineralized silk fibre lay completely, allowing the manufacturing process to be enormously simplified. Biocompatibility of the silk matrix in both mineralized and unmineralized states was demonstrated on WP's 6, 7, and 8.

The only outstanding question with respect to Strategic Objective 2 regards the osteoinductive capacity of the material. Data presented from both WP7 and WP8 clearly demonstrates the osteoconductive nature of SilkBone™ BSM, i.e. the capacity of the material to support the attachment of new osteoblasts and osteoprogenitor cells by providing an interconnected structure through which new cells can migrate and new vessels can form. Osteoinductivity – the capacity to induce non-differentiated stem cells or osteoprogenitor cells to differentiate into osteoblasts – was not established in entirety. In general osteoinductivity requires an active biological agent such as Bone Morphogenetic Protein (BMP) to be present, and early in the project a cost-benefit analysis and freedom to operate survey established that purchase of commercial BMP and incorporation into the SilkBone™ BSM was not a feasible commercial route for the project. While an 'in-house' source of BMP was

manufactured during the course of the project, which represents a collateral commercial outcome for SME Partner 3H biomedical, this was not incorporated into the final iteration SilkBone™ BSM for both strategic and timing reasons.

However, data presented in WP7 and WP8 shows that osteoblast cells are both recruited to the SilkBone™ BSM *in vivo* and osteogenic progenitors correctly differentiate into osteoblasts and osteoclasts. Though further testing of the material is required to conclusively demonstrate osteoinductive capability of the SilkBone™ BSM these represent extremely positive initial findings.

Strategic Objective 3

This was fully completed, successfully and in entirety and is reported on at length in WP7 and WP9 in Periodic Activity report 2. Stem cells were successfully isolated from bone marrow of consenting donors by both Partners Southampton University and Progentix B.V. These were successfully seeded on final iteration SilkBone™ BSM and culturing demonstrated growth and proliferation of cells on the scaffolds and ingress of cells into the porous structure. In addition, Partner Progentix also developed advanced stem cell bioreactor technology for amplification of patient own stem cells and conducted cost-benefit and regulatory analyses for the use of this technology commercially.

8. Achievement of Project Milestones

The SilkBone project was structured around the achievement of 12 time related milestones, listed on Page 4 of the SilkBone Description of Work and reproduced below (***text in bold blue italics***). Following each a brief report on the final status of the Project Milestone is provided in terms of whether and when it was successfully completed, modified or abandoned.

6 months:

1. *"Best" processed silk selected.*

This Milestone was achieved on time. Wild silk fibres of the *Antheraea* genus were selected from over 15 species of silk tested and successfully processed on WP1 to achieve significant mechanical strength as reported on WP5, biocompatibility as reported on WP6 and WP8 and were supportive of cell growth as reported on WP7.

Silk fibroin solutions were successfully generated on WP2 with the best properties achieved using silk from the *Bombyx* genus. Similarly, matrices formed from silk fibroin solutions were processed on the other WP's to establish mechanical and biological performance data.

Respectively, these were selected for use as fibre and matrix components.

2. *Pilot quantities of porous osteoinductive composite*

This Milestone was completed on time with the exception that osteoinductivity was not conclusively demonstrated due to the decision not to include a source of Bone Morphogenetic Protein (BMP) or other osteoinductive agent in the SilkBone material, as described in the report on Strategic Objective 2 in the previous section. Mineralised composites of hydroxyapatite with both silk fibres and regenerated silk matrices were produced by six months. Porous fibre lays were generated on WP3 and preliminary methods for production of prototype porous regenerated silk matrices were developed on WP2 and these were then mineralized by processes developed by Partner Bristol University on WP4.

3. *Mineralised composite with satisfactory though non-optimised mechanical properties.*

This Milestone was completed on time. Mineralised composites of hydroxyapatite from Milestone 2 were examined and following physical and chemical examination on WP4 and mechanical evaluation on WP5 a decision was eventually taken to focus mineralized regenerated silk matrices without incorporation of a fibre lay which was considered largely redundant given the excellent early compressive properties obtained in these mineralized regenerated silk fibroin matrices and the added manufacturing complexity of introducing fibre lay into the production process

12 months:

4. *Optimised fibre lay.*

This Milestone was rendered obsolete as a decision was taken not to incorporate a fibre lay in the final iteration material. Nonetheless, nine types of fibre lay were generated on time in WP3, two of which were subsequently employed in prototype medical devices – a heartvalve and a suture thread - by Partner Oxford Biomaterials (described in PAR2, WP3 report). The Suture thread has subsequently attracted investment finance for commercialization.

5. *Pilot quantities of first generation cell-seeded composite available for implantation into Rats.*

This Milestone was achieved on time. Porous mineralized silk fibroin matrices were provided to Southampton University for cell seeding on WP7 and demonstrated excellent results in *in vitro* cell culture assays. Immuno-supressed nu/nu mice were substituted for Wistar rats in the trials on WP8 so that composites could be seeded with human cells and implanted.

6. Schedule defining guidelines for regulating bone stem cell expansion

This Milestone was achieved on time. A report compiled by an independent regulatory consultant on the likely regulatory requirements and strategies required for proceeding with patient own cell expansion therapies for bone repair was commissioned by Partner Progentix and was provided in PAR1, WP9.

15 months:

7. Cytokine assay predicts mineralised composite likely to be well tolerated

This Milestone was achieved on time. In line with the EU directive to reduce refine and replace animal experimentation, Partners InPuT and 3H both performed a series of cytokine profile assays with repeated iterations of mineralized of silk fibroin scaffolds, all of which demonstrated that the scaffold materials were likely to be well tolerated once implanted in the human body. Final iteration cross linked mineralized silk fibroin scaffolds was also shown to be well tolerated, and is reported on in WP6, of PAR2, although this was not completed until month 24.

24 months:

8. Optimised porous osteoinductive composite.

This Milestone was achieved on time with the exception, as detailed above in the report on Strategic Objective 2, that osteoinductivity had not been conclusively demonstrated. An optimized porous mineralized silk fibroin scaffold was produced by Month 24 samples of which were subsequently demonstrated to be osteoconductive and well tolerated in *in vivo* implantation experiments on WP8. These experiments also demonstrated the composite remodeled through the action of osteoblasts and osteoclasts, and recruited osteogenic cells to the implant site.

9. *Optimised heavily mineralised composite capable of load bearing when implanted.*

This Milestone was achieved on time. The optimized porous mineralized composite described above was shown on WP4 to have a proportion of hydroxyapatite nanocrystals that could be varied between 30% and 70% of the total scaffold. Samples of these demonstrated the requisite mechanical tolerances set out in WP5 of the SilkBone Description of Work, achieving in excess of 20 MegaPascals (MPa) of compressive load in some examples and an average of 14MPa.

10. *Mechanical data predict composite will bear load when implanted.*

This milestone was achieved on time. The final iteration composite scaffold reached by month 24 and described above, presented sufficient mechanical tolerance to be considered load bearing in bone repair indications. As the mechanical data recorded on WP5 were achieved in a hydrated state as well as dry state, it is predicted that the load bearing capability would be translated into the *in vivo* environment.

11. *Proof of concept of safe and effective bone graft material*

This milestone was achieved on time with the exception that one stringent safety test of the material on WP8 was not passed succcessfully. This is thought to be due to the current non-optimised manufacturing capabilities and should be rectified once GMP manufacturing standards have been put in place. All other safety and efficacy standards set out in WP's 5, 6, 7, and 8 were achieved, and the final iteration composite scaffold demonstrated osteoclastic remodeling in *in vivo* experiments on WP8, indicative of being an effective bone graft material.

12. *Proof of concept of stem cell expansion and differentiation into functional bone cells.*

This milestone was achieved on time. Partner Progentix developed bioreactor technology on WP9 which demonstrated effective amplification of human derived adult stem cells, and, together with Partner Southampton on WP8, demonstrated that human derived cell populations could adopt functional bone cell lineages both *in vitro* and *in vivo*.

8. Achievement of Project Key Success Measures

The key scientific and technological, public, and commercial success measures of the SilkBone project are listed on pages 22 to 24 of the SilkBone Description of Work and are reproduced below (*text in bold blue italics*). Following each, a short description of whether these expected benefits were attained is provided:

i. Key Scientific and Technological Benefits.

The following key commercial scientific and technological benefits to be generated within the project duraction.

1. *High performance generic resorbable biomaterial fibre with criteria specified in WP1.*

This was achieved. Although a fibre based biomaterial was not ultimately included in the final iteration SilkBone scaffold, a successful silk biomaterial fibre was developed on WP1, and subsequently proven to be resorbable in experiments carried out with a collaborator not on the SilkBone project and not using SilkBone project resources (it being reasoned that as the material was not going to be included in the

stated Strategic Objectives of the SilkBone project, SilkBone resources should not be expended on its further development). The criteria set out in WP1 are:

WP1 Criteria

i. Tensile properties of fibre in excess of those of demineralised bone (type I) collagen (toughness 13kJkg^{-1}; ultimate tensile strength 0.1 GPa) .
ii. Tensile properties in excess of those of commercial mulberry silk (toughness 60 .kJkg^{-1} ; ; ultimate tensile strength 0.45 GPa)
iii. Batch to batch consistency in these properties better than 15%.
iv. Absorption time 2-9 months depending on application.
v. Silk can be safely heat sterilized with less than 10% deterioration in assessed tensile properties.
vi. A biocompatible cross-linking method required for stabilizing the matrix protein does not produce more than 10% reduction in assessed tensile properties.

Tensile properties of 90kJkg^{-1} and 0.49GPa were achieved with a batch to batch consistency of less than 5% variation observed meaning criteria 1, 2 and 3 were successfully achieved. In the subsequent *in vivo* implantation trials a resorption half life of 3 - 6 months was observed depending on the formulation of the material implanted, indicating that criterion 4 was achieved. Heat sterilization was found to be effective with no measurable deterioration in mechanical properties, and subsequently outside the remit of the SilkBone project, the fibres were found to be effectively sterilised with gamma irradiation as well, indicating criterion 5 had been met. The cross linking reagent used to stabilise the final iteration SilkBone matrix material was found to increase the assessed mechanical properties considerably meaning criterion 6 was also achieved satisfactorily.

2. *Porous resorbable osteoinductive mineralised composite with criteria specified in WP2, WP3, WP4 and WP8.*

The objective of achieving a porous resorbable mineralised composite was achieved. The majority of the criteria specified in WP's 2, 3, 4 and 8 were achieved in full, although due to changes in project design, in particular with respect to not

including a source of BMP in the SilkBone scaffold, meant that certain criteria were rendered obsolete or were met only in part. The criteria in the SilkBone Description of Work for these work packages are as follows:

WP2 Criteria:

100g wet weight of an optimised porous mineralized and osteoinductive material suitable for loading with mesenchymal cells. Optimised shall mean:

i. ***pore size will be 10 to 50 µm (for optimal cell ingress) and pore density 5-20% of cross sectional area.***
ii. ***an ability to stimulate bone formation better than that of collagen sponge impregnated with a standard dose of rhBMP-2.***
iii. ***The composite having a resorption time of 2-9 months when implanted into the abdominal cavity of rats .***

Several hundred grams of material were produced as part of final iteration processing and supplied to consortium partners for evaluation.

(i) Pore sizes of 10 – 50 microns were achieved although it was found that a heterogenous pore size with an average size of around 100 microns yielded better results in terms of cellular ingress, and this was chosen for the final iteration material. A revised examination of the literature in the area supports 100 microns and above as being a more appropriate pore size. Porosity could be varied in the scaffold considerably, and the described porosity was achieved, although a porosity more similar that of human trabecular bone (70%) was employed in the final iteration material. The capacity to increase both the pore size and the porosity in the final iteration material to that found in human trabecular bone whilst retaining excellent mechanical strength was unexpected and allowed the consortium to exceed the specified criteria in these areas.

(ii) As a source of rhBMP was not included in the final iteration material due to the reasons elsewhere explained of cost and freedom to operate, this criterion could not be assayed. However, promising demonstrations of scaffold remodelling were

observed on WP8 indicating the capacity of the material to induce bone formation when in the presence of osteogenic cells.

(iii) Long term implantation studies were not possible within the timeframe of the project due to delays in achieving the optimised material, but these studies are currently underway at Visionar A.B. an MDA and FDA approved facility in Uppsala, Sweden subcontracted by Partner SLU. Preliminary results have been encouraging and the remodelling of the scaffold demonstrated by Partner Southampton on WP8 indicated that the criterion is likely to be met. The realisation that these studies would not be completed within the timeframe of the project led to *in vitro* enzymatic absorption assays being carried out on iterations of the SIlkBone scaffold by Partner Bristol which also indicated that the absorption criterion was likely to be achieved, and are reported on in WP4.

WP3 Criteria:

Ten specimens of a fibre lay which after incorporation of matrix and subsequent mineralization prove suitable for implantation as an experimental bone graft substitute or aid to bone healing.

Nine specimens of fibre lay were generated on WP3 which were considered suitable for incorporation into Bone graft materials before the decision was taken by the consortium that a fibre component would not be required in the final iteration SilkBone bone substitute scaffold due to the excellent results obtained with mineralised composites using optimised regenerated silk fibroin solutions. Efforts to mineralise these were undertaken on WP4 but also rendered largely obsolete by this decision. As reported in PAR2 WP3 report, these fibre lays were successfully used on other projects including a composite prototype bone fixation plate, a composite nerve regeneration guide, and a braided fibre suture all of which are currently being developed with industrial partners.

WP4 Criteria:

An optimised mineralised silk composite with compressive or bending strengths, moduli and toughnesses either better than competitive materials or within 10% of the values for cadaveric bone at the target site(s) for implantation.

The final iteration SilkBone Scaffold formed from mineralised regenerated silk fibroin achieved mechanical tolerances comparable with lumbar bone, the most likely initial implantation site and over 3 times the compressive strength of any currently available resorbable bone substitute material.

WP8 Criteria:

The evidence presented in the report [on long term implantation studies] is of sufficient quality to be submitted directly to the FDA as part of their requirement for evidence of safety and efficacy in lower mammals.

The implantation work of the final iteration SilkBone scaffold material is being performed by Visionar AB, an FDA and MDA accredited facility, and the report that will be compiled following long term implantation will be to the standards required for submission as part of the dossier for the regulatory approvals process. The work was subcontracted from Partner SLU to Visionar in part because of the recommendations from PAR1 that the consortium engage more fully with the requisite regulatory processes. By moving to an accredited facility with dedicated QA procedures and a deep understanding of the compliance required for medical device approvals the project resources were felt to be better deployed than in the original experimental procedures outlined in WP8 in the Description of Work.

3. A cellular bone graft material with compressive properties in excess of those of competitors (criteria specified in WP1, WP2, WP3, WP4 & WP5).

The SilkBone final iteration bone graft material was successfully seeded with cells both *in vitro* and *in vivo* in nude mice and in this state was capable of retaining full mechanical properties described which are approximately three times as high as

those of competitor's absorbable bone graft material. The evaluatory criteria specified in the Description of Work for WP1, 2, 3 and 4 have been described in key success measures 1 and 2 above, and the criteria for WP5 are given below:

WP5 Criteria

Mechanical data are of sufficient quality to assess whether the best mineralized composites are either better than competitive materials or can be used for an orthopaedic application either as a coat for metallic prostheses or as a bone graft substitute or for augmentation of bone repair.

The mechanical data reported in PAR2 WP5 clearly indicate that the final iteration SilkBone material is both greatly in excess of current competitors materials, and of sufficient strength to be used for orthopaedic applications as a bone graft substitute, and, given a favourable reception from the surgical community, for augmentation of bone repair as well. In addition, the failure mode of the SilkBone material is non-catastrophic, and its capacity for ductile deformation which can be seen in Figure 3 of this report suggest that even when mechanical tolerances have been exceeded the material will mould to fit surrounding bony tissue. Consequently, whilst the material in its current form is not suitable for coating metallic prostheses, it may be well suited to use in impaction grafting and for packing around metallic implants in joint arthroplasties and revision joint arthroplasties where load bearing material is required and acts in part to prevent the prosthesis from working loose.

4. *Method of maintaining and expanding adult mammalian stem cell culture as specified in WP9.*

Bioreactor technology developed by Partner Progentix which is reported on at length in PAR2 WP9 and illustrated in Figure 4 of this report is fully capable of expanding human populations of adult stem cells derived from patients own bone marrow.

5. *Schedule for defining guidelines for regulating the industrial expansion of human adult stem cells (WP9).*

The required schedule defining these guidelines was produced as part of the report on PAR2 WP9 and provides an in depth analysis of the regulatory, financial, ethical and technical requirements for the expansion of human adult derived stem cells.

6. *Cost/benefit analysis of method for expanding adult mammalian stem cells (WP 9).*

The Bioreactor technology developed by Partner Progentix which is reported on in PAR2 WP9 includes an entire WP objective (9.5) which details a cost analysis for the industrial implementation of a system for expanding patients own bone marrow stem cells for use in bone repair applications. The outcome of this detailed analysis was that a system could be considered at a current cost of EURO2137 per patient, of which hardware costs were EURO650, The disposable Bioreactor costs were EURO1281 and consumable medium costs were EURO205. Partner Progentix are evaluating market conditions and surgeon perspectives with respect to launching the system. The benefit of a patient own cell system is clear in morbid indications and in patients in which bone stock is seriously depleted, but less so in patients where bone lesions are small and bone stock is healthy. As a result a thorough market analysis is required to ensure the product is targeted at the appropriate market sectors, and this is being conducted by Partner Progentix pending he launch of their disposable bioreactor system.

7. *Method for successfully incorporating adult stem cells into porous mineralised composite (using criteria specified in WP7).*

Initially, problems were encountered by Partners Progentix and Southampton in seeding bone substitute materials developed by the consortium, with efficiency of

seeding comparatively low. The causes of this were due to the fact that seeding protocols used had been defined for other systems which proved not to translate optimally for SilkBone materials. It was found that vacuum infiltration of SilkBone materials with culture medium followed by light centrifugation of the SilkBone material with the cells provided the best method for cell seeding.

8. *Dose response curves for different slow release formulation of rh-BMP-2 (WP7).*

As detailed above, the decision of the consortium not to include BMP in the final iteration scaffold materials due to the cost and freedom to operate implications resulted in success measures with respect to BMP release from the scaffold being rendered obsolete.

9. *Prediction of commercial viability of rh-BMP-2 incorporation into bone graft substitutes (WP7).*

An investigation of the commercial viability of incorporation of recombinant human BMPs into the SilkBone bone graft substitute was conducted by consortium members early in the project, and, as reported in PAR1 at month 12, it had been decided that incorporation of a commercially sourced BMP in the scaffold material was not a feasible commercial reality due to potential infringement of existing patents and the cost/benefit of including BMP in the scaffold material. A source of in-house rhBMP was generated during the course of the project by Partner 3H, and may be included in future generations of SilkBone products, but the current comparatively low cost of competitors bone graft materials and difficulty with freedom to operate issues meant inclusion of BMP was not considered viable.

10. *Evidence from in vitro and in vivo testing of safety and efficacy of bone graft substitutes of a quality and in a format suitable for submission to regulatory bodies.*

Safety testing of SilkBone bone graft material is being conducted by Visionar A.B., an accredited preclinical medical devices testing organisation, and reporting is to FDA and MDA standards for subsequent incorporation in the product history technical file. To date the material has passed all safety testing with the exception of one stringent test which is believed to be due to non-optimised production methodology. This will be rectified once production is conducted under GMP protocols.

Predicted patent applications arising within the time frame of the project.

The following inventions arising from the scientific and technological benefits will be considered for patent applications. It is likely that between five and seven patent applications will be chosen from the prioritised list given below:

1. *Apparatus and method for producing high performance generic resorbable biomaterial fibres.*
2. *Apparatus and method for producing porous resorbable implantable materials.*
3. *Apparatus and method for producing porous osteoinductive mineralised composite.*
4. *Apparatus and method for producing bone graft substitute materials.*
5. *Method of maintaining and expanding adult mammalian stem cell culture as specified in WP9.*
6. *Method for incorporating adult stem cells into porous mineralised composite (using criteria specified in WP7).*
7. *Method for delaying the in vivo release of osteogenic factors.*

8. Methods for improving the tensile properties and delaying the resorption of generic resorbable fibres and composite.

Four patents were filed directly as a result of the efforts of the SilkBone consortium, and it is expected that two further filings will be made once the outcome of ongoing work, initiated on the SilkBone project, has been completed. In addition, there has been a considerable number of new methodologies developed in the areas of silk processing which are not suitable for patent filings, either because of the impossibility to enforce and police them or because there are questions over the whether they would be granted which will remain as 'black box' know-how. Patents protecting the results of the SilkBone project which have been filed to date are:

1. Implantable cartilaginous tissue repair device: WO/2007/020449
International filing date: 17/08/2006
Priority date: 17/08/2005
Applicant: Oxford Biomaterials

The core teachings of this patent application relate to the use of porous silk scaffolds in the repair and regeneration of cartilage and their anchorage into bony substrates by mineralized porous silk scaffolds.

2. Tissue regeneration; US patent application, Application number 11/298,208; Publication number US2006/0136068;
Priorty date: Dec. 8, 2005.
Applicant: Progentix B.V.

The core teachings of this patent application are centred on the postponed application of stem cells in biomaterial scaffolds for bone tissue engineering.

3. Cartilage Repair Material and a method of preparation thereof: GB 0807868.5

National filing date: 30/04/2008
Priority date: 30/04/2008
Applicant: Oxford Biomaterials

The core teachings of this patent application are focused on methods for the production of cartilage repair materials using optimized silk fibroins.

4. Bone Repair Material and a method of preparation thereof: GB 0811542.0
National filing date: 24/06/2008
Priority date: 24/06/2008
Applicant: Oxford Biomaterials

The core teachings of this patent application are focused on the development of novel mineralization techniques and cross linking methods in the production of silk fibroin-hydroxyapatite bone substitute materials,

These patents cover intellectual property described in items 1, 2, 3, 4, 5, and 8 of the list in the SilkBone Description of Work reproduced above. Item 6, (*Method for incorporating adult stem cells into porous mineralised composite)* involved use of methodology that was not considered unenforceable even if the protocols were considered sufficiently novel and inventive to warrant patenting, and item 7 (*Method for delaying the in vivo release of osteogenic factors)* describes methodology that, in the event, was not explored in the project. However, in addition to the 8 items foreseen in the Description of Work, patents relating to both fibrous cartilage repair and articular cartilage repair have been filed by the consortium due to the development of scaffolds from optimized regenerated fibroin which have potential in meniscal cartilage repair, spinal disc repair, and articular cartilage and osteochondral applications.

ii. Public benefits arising within the time frame of the project.

1. *Academic publication areas as defined below.* **(See Below)**

2. *Attendance and participation in at least four conferences. This includes presentation of abstracts, posters and talks.*

Consortium members represented the SilkBone consortium at 3 international conferences: EuroBio 2007 in Lille, France, EU-TERMIS 2006, Rotterdam, and European Symposium for Biomaterials 2006, Nancy, France. Both abstracts and oral presentations were made. In addition, the consortium was represented at National level conferences, such as the British Orthopaedic Research Society Meeting 2006, and the Bionis BioInnovators meeting at the University of Reading, 2008. During the second reporting period as interest in the SilkBone project gathered due to the dissemination activities during the first reporting period, the project coordinator received a large number of invitations to present the Project at various events. Events were chosen judiciously, with an aim to give a satisfying presentation for the target audience and raise the projects profile without providing a level of detail that would compromise intellectual property. One eye was also on presenting to as broad a spectrum of audience as possible. These events chosen were therefore at financial institutions (SilkBone Investor Presentation, Library House Ltd. March 2007), Universities (Plenary Speaker at the University of Nice, March 2007), academic societies (Novel Bone Substitutes, Royal College of Surgeons, November 2007) industry trade fairs (Orthox – a spin out vehicle for the SilkBone project, Biotrinity, April 2008) research conferences (SilkBone Partnering Meetings, EuroBio October 2007) European focus groups (Bone Tissue Engineering, BioSmile, October 2007) and entrepreneurial events (Orthox – a spin out vehicle for the SilkBone project, VentureFest, July 2008) and have been well received.

3. At least three talks to special interest groups.

During the second reporting period as interest in the SilkBone project gathered due to the dissemination activities during the first reporting period, the project coordinator received a large number of invitations to present the Project to special interest groups at a number of different events. Events were chosen judiciously, with an aim to give a satisfying presentation for the target audience and raise the projects profile without providing a level of detail that would compromise intellectual property. One eye was also on presenting to as broad a spectrum of audience as possible. Presentations were made to financial institutions (SilkBone Investor Presentation, Library House Ltd. March 2007), Universities (Plenary Speaker at the University of Nice, March 2007), academic societies (Novel Bone Substitutes, Royal College of Surgeons, November 2007) industry trade fairs (Orthox – a spin out vehicle for the SilkBone project, Biotrinity, April 2008) research conferences (SilkBone Partnering Meetings, EuroBio October 2007) European focus groups (Bone Tissue Engineering, BioSmile, October 2007) and entrepreneurial events (Orthox – a spin out vehicle for the SilkBone project, VentureFest, July 2008) and have been well received.

4. At least four press-releases.

Two press releases publicising research outputs of the SilkBone project have been made, the first relating to the production of spider-like silks produced by Partner Oxford Biomaterials on WP1, and the second relating to the formation of the spin out venture Neurotex, which is developing those spider-like silks for neural repair. These were disseminated to a large number of press organisations Europe-wide through the use of Alphagalileo, a subscription based press agency and can be found on the Oxford Biomaterials website (www.oxfordbiomaterials.com).

Three further press releases are planned focussing specifically on the major outputs of the SilkBone project. The first will be released in late September to announce the major outputs of the SilkBone project and the launch of the funding drive for Orthox Ltd., the commercialisation vehicle for the SilkBone cartilage and bone repair materials. The second press release will be issued following the publication of the projects key article in a major international journal "Bone-like resorbable silk-based scaffolds for load-bearing osteoregenerative applications" which was submitted to Nature Materials for peer review in June 2008. Finally a third press release will be issued following successful generation of funding for Orthox Ltd. to commercialise the outputs of the SilkBone project. Press releases will be issued through Alphagalileo and also psotd on the SilkBone website (www.silkbone.org), the Oxford Biomaterials website and the newly launched Orthox website (www.orthox.co.uk).

5. At least two articles in wide circulation newspapers/ periodicals.

One article relating to the outputs of the SilkBone project entitled 'Weaving Webs of Wonder' has appeared in the widely read newspaper, Oxford Today (2007 readership, 150,000, http://www.oxfordtoday.ox.ac.uk/2007-08/v20n2/04.shtml) and a further article will appear in the Oxford Times (2007 readership, 100,000). This will detail biomimetic solutions, such as the SilkBone approach, to solving engineering problems, and will appear in the September edition of the paper. The consortium also hope to gain national coverage in the broadsheet newspapers in the event that Orthox receives funding, and will be making contact with science editors following the issue of the planned press releases. There is also interest from the widely read periodical, New Scientist, in a feature on SilkBone and other uses of silk in tissue repair which it is hoped will lead to an article during the autumn of 2008, to coincide with the Orthox funding drive.

6. At least one article in a trade magazine.

Two articles detailing the outputs of the SilkBone project have appeared in trade magazines. The first of these, entitled 'Silky Feeling' appeared in Materials World in summer 2007 detailing nerve repair featuring the silk fibres generated on WP1 of the project (http://www.iom3.org/materialsworld/aug07/feat.htm). The second article appears in the August edition of Medical Device Developments and focuses on the SilkBone Bone repair and Cartilage repair materials developed on the Project.

7. At least two national radio/ television appearances.

Dr David Knight of Partner Oxford Biomaterials featured on the UK National Radio 4 programme, Materials World, hosted by Dr Quentin Cooper, discussing the SilkBone project and other applications of spider-like silks for biomedical tissue repair. It is hoped that this will be followed up with a further appearance on the programme this autumn to discuss the successful achievement of the projects objectives and the launch of the commercialisation vehicle, Orthox Ltd.

Orthox and the SilkBone technology will also be featured in the forthcoming publication 'Natures 100 Best - World Changing Innovations Inspired by Nature' by Gunter Pauli and Janine Benyus which will be released in October 2008. The authors are two of the world's foremost thinkers on biomimcry and the low carbon economy. Gunter Pauli founded the Zero Emissions Research Institute (ZERI, www.zeri.org) and was the entrepreneur who founded the Ecover range of products. The aim is to launch the publication not only to the general public, but also to target it at high level individuals in the worlds of finance, industry and policy to effect maximum impact. As a featured technology in Natures 100 Best, SilkBone will be exposed to a wide and high level audience and this may lead to further invitations to present the technology on television and radio.

8. *Website to be functional within three months of the start of the project and to be updated at least bimonthly.*

The Project website was established by month 3 of the Project and can be accessed at URL www.silkbone.org. Other domain identities www.silkbone.com and www.silkbone.eu have also been reserved. The website was most recently updated in August 2008 with final results of the project, and will continue to be maintained for at least a further 3 years to ensure that the projects results are suitably disseminated and to provide contact details for receiving further information about the project and the ongoing progress of its outputs. The website is now complemented by a website dedicated to Orthox Ltd. at www.orthox.co.uk, the commercialisation vehicle for the projects results, which was launched in August 08.

9. *Published schedule for defining guidelines for regulating the industrial expansion of bone marrow stem cells.*

This schedule forms part of the write up of WP9 and provides a detailed description of the requisites for expansion of human bone marrow stem cells. Currently a suitable vehicle for publication of this report has not been determined.

Predicted areas for academic publications submitted within the time frame of the project.

1. *Properties of Spidrex fibres.*
2. *Cross-linking silk fibres and composites*
3. *Properties of generic porous composites.*
4. *Mineralisation of silk fibres and composites.*
5. *Mechanical properties of mineralised composites.*
6. *Cytokine profiling of silk-based fibres and composites.*
7. *Effects of delayed release formulations containing osteogenic factors.*

8. Biocompatibility and resorption rates of silk fibres and composites.

9. Expansion of adult stem cell cultures

10. Osteogenic differentiation of adult stem cells.

11. Incorporation of osteogenic cells into mineralised composites.

12. Efficacy of mineralised composites in vivo.

The academic publications listed in the project dissemination plan below cover all the subject areas listed above with the exception of item 7, *Effects of delayed release formulations containing osteogenic factors.* This was due to the fact that a study of osteogenic factors was not conducted on the project.

Paper numbers and titles cannot be predicted ahead of the research but target of 10 key papers in high impact journals has been set. Papers will only be submitted when the related intellectual property has been adequately protected.

As detailed above, a number of project publications were delayed until intellectual property was adequately protected by the patent filings 'Bone repair material and a method of preparation thereof' and 'Cartilage repair material and a method of preparation thereof'. Nonetheless, the target of 7 publications in high impact journals have been published, and a further 3 papers have been submitted for peer review which are listed below in the dissemination plan. Provided these papers are successfully published, the target of 10 publications will have been met.

iii. Commercial Benefits

We plan to achieve the following sequentially-linked commercial benefits within the time frame of the project:

1. *Ample scientific publications and wider media coverage (as specified in the dissemination plan) to interest potential investors and other senior business partners.*

Whilst there has been a close control over the publication of data and and interation with the broadcast media through the desire to protect intellectual property generated by the project, scientific outputs from the SilkBone project have already led to the acquisition of £500,000 in equity finance for the spin out vehicles Neurotex Ltd. and Suturox Ltd. and have led to collaborations for a bone fixation plate and cartilage repair technologies with two major international business partners (who cannot be named in this publishable report due to the stipulations of confidentiality agreements that have been put in place). Significant interest from the investment and industrial communities has also been expressed in Orthox Ltd., and it is expected that this will increase as further articles detailing the SilkBone project and its outputs are published.

2. *Protected intellectual property arising from the project.*

Four patents, detailed above, have been filed as a result of data produced on the SilkBone project. In addition, the trademark 'Spidrex®', covering the generic silk technologies produced on the project, has been registered in the UK.

3. *High quality data for use in obtaining statutory approval.*

The production of data required for obtaining statutory approvals in the regulatory process for the SilkBone materials is ongoing with Visionar A.B., an accredited pre-clinical trials company conducting the tests according to the ISO standards. Visionar were engaged as a subcontractor in response to the project mid term recommendation that the consortium engage more fully with the regulatory process, and processes have been planned and supervised by a

qualified medic (Urban Hogland), a regulatory specialist (Gunilla Nyberg) and a quality assurance manager (Annika Grahn-Westin). The final package of data has not yet been completed, but will be of sufficient quality for submission to the regulatory bodies, which is likely to be a notified body for the competent authority in Sweden.

4. Formation of a company to exploit the new technology and act as a vessel for investment.

A company, Orthox Limited (www.orthox.co.uk), has been incorporated and registered in the U.K. with company number 6606448. Orthox Ltd. is the intended commercialisation vehicle for the SilkBone Bone Substitute Materials and Cartilage Repair Materials generated by the project.

5. High quality business plan based on the technology developed in the project.

A full business plan for Orthox has been prepared by the Project Coordinator, Dr. Nick Skaer of Partner Oxford Biomaterials who has joined Orthox as Chief Executive and once investment in the company has been secured will leave his position as CEO of Partner Oxford Biomaterials to manage Orthox full time. The business plan has also had significant input from several leading experts who have also signed contractual terms with Orthox and will take up salaried management and board positions once investment has been secured. These individuals are Mr Michael Nairn, a Financial Consultant for Management Edge Ltd., who will join Orthox as Finance Director; Professor Christina Doyle, an orthopaedics industry expert, who will Chair the Orthox Board of Directors; Professor Oliver Kessler, a Zurich based knee surgeon who will join Orthox as Chief Medical Officer, and Mr Antony Odell of Xenomedical Ltd., an orthopaedics regulatory and marketing expert who will advise on strategy in these two areas.

An Executive Summary for Orthox is appended to this report and a copy of the full business plan is included in PAR2.

6. *Preliminary approaches to venture capitalists and senior business partners in orthopaedic materials. Whilst precise costing to take the project through to commercialisation will only be defined during preparation of the Business Plan, a preliminary target figure of €3.000.000 is considered realistic at this stage.*

Whilst equity capital and industry partners have been approached, Orthox is hoping to secure initial funding from the UK based biomedical charity, the Wellcome Trust. An application for £1.6 million (€2 million) has been submitted and has progressed to the final judging round, with a decision expected on September 5th 2008. A further £2 million (€2.5 million) will be sought from investors, and preliminary approaches for securing private equity investment capital to fund Orthox have been made to U.K. based venture capitalists Exomedica, Technikos, Spark Ventures, Advent, and Oxford Technology. However, a full funding drive will not be launched until the decision from the Wellcome Trust has been received as this will significantly affect the company's valuation.

7. *In addition to the long term commercial goal of producing a resorbable BSM, we have identified the following market sectors for rapid penetration from generic technological innovations arising within the project:*
    - *Cell and tissue engineering scaffolds*

The generic silk cell and tissue engineering scaffolds which were developed on WP2 proved to have unexpectedly excellent mechanical properties due to the optimised silk fibroin solutions employed in their construction. Their profound resilience and

compressive strength indicated that they were clearly very well suited to use as cartilage repair materials – a very large potential market sector in which there are currently very few successful tissue regenerative products. The Cartilage repair materials form part of the product portfolio of Orthox Ltd.

- *Hernia meshes*

An investigation of the potential for a hernia repair material using the fibre lays generated on WP3 was investigated with collaborators at the University of Fudan in China. However, an assessment of current market conditions did not indicate that a development should be pursued at this point. In addition, the cell binding attributes of the 'RGD' sequences present on the Spidrex® fibres developed on the SilkBone project, suggested that a problem with adhesions between the mesh and tissue might develop. However, fibre lays developed on WP3 have been employed in the development of a Spidrex® sututre thread and a Spidrex® nerve repair conduit, both of which have successfully attracted equity finance for commercialisation.

- *Dermal patches*

Dermal patch technology was developed on WP2 and reported on in PAR1 as part of the development of the silk scaffold technology. A prototype product – a transparent pure silk fibroin patch with 200% extensibility and excellent tensile strength was developed. The technology was showcased to a major international medical device company under the product name DermaSilk™ but was considered too early stage to be taken further as part of a development collaboration at this stage. The product will look to be developed with further public funds in the future.

- *Vascular stents*

Fibre lays developed on WP3 were combined with optimised fibroin films developed on WP2, and other matrix materials such as silicone to create tough composite materials. Together with a cardiac surgeon at Great Ormond Street Hospital these composites were fashioned into a series of prototype vascular stents and heartvalves. The development remains at an early stage but there is clear potential for the technology in this area and with the available surgical expertise on the project the prospects for development appear good.

10. Final Status of Project Deliverables

WP No.	Deliverable No. & Deliverable Name.	Delivery Date	Person Months	Lead Participant	Nature	Dissemination level	Status
WP1	1. Report on the tensile properties of different natural silks and consistency from batch to batch.	6	3	5	Report	Public	Completed on schedule
	2.Report on effects of heat and cross-linking agents on tensile properties.	6	3	5	Report	Confidential	Completed on schedule
	5. Reports on the effects of cross-linking agents on absorption rates of silk filaments and the predicted bio-compatibility in conjunction with WP6.	18	3	1	Report	Confidential	Modified deliverable completed on schedule
	3. A suitable silk for incorporation into bone graft replacements.	6	3	1	Demo	Confidential	Completed on schedule
	4. A method of heat sterilizing this silk without drastic reduction in mechanical properties.	6	4	1	Demo	Confidential	Completed on schedule

WP2	7. A composite material with suitable resorption rate, micro porosity for cellular ingress, and optimized capacity to stimulate bone formation.	19	20	1	Demo	Confidential	Completed on schedule
	8. A report detailing the effects of cross-linking agents on biocompatibility and resorption time will be written in conjunction with WP7.	30	12	7	Report	Confidential	Completed on schedule
	9. A report detailing the effect of different initial concentrations of rhBMP-2 and methods of incorporation on its rate of release (in conjunction with WP6) and on the extent of the osteoinductive effect (in conjunction with WP7 and 8).	30	12	4	Report	Confidential	Deliverable abandoned
WP3	6. Fibre lays with appropriate properties for use as an interfacial material surrounding a metallic prosthesis and for a bone replacement/repair material.	19	12	1	Demo	Confidential	Completed on schedule
WP4	10. Hydroxyapatite mineralized composite with sufficient mineral density to permit	30	50	6	Prototype	Confidential	Completed on schedule

	load bearing when implanted 11. A report detailing the methods used to prepare mineralized silk composites; assessments of the mineralization of these; and the relationship between the extent of mineralization and matrix particle size on the load-bearing properties of the mineralized silk composites.	30	4	6	Report	Public	Completed on schedule
WP5	12. A report giving mechanical test data to show that prototype implantable composites will bear load at implantation and at a range of times after implantation.	30	24	7	Report	Confidential	Completed on schedule
WP6	13. Report on the cytokine release profile and toxicity to cells produced by exposing human whole blood to implantable materials produced by the consortium.	12 & 30	40	3	Report	Public	Completed on schedule
	14. Report on the rates of release of rh-BMP-2 from various modifications of Spidrex matrix (see WP2).	24 & 30	23	4	Report	Public	Deliverable abandoned

WP7	15. A report defining what the extent and quality of cell adhesion, cell viability, cell proliferation, osteoblast differentiation and bone formation is *in vivo* and the optimal conditions for this.	24 & 30	24	7	Report	Confidential	Completed on schedule
WP8	16 comprehensive report on the safety and efficacy of bone graft replacement materials produced by the consortium for use in the subsequent approvals process.	30	21	8	Report	Public	Deliverable delayed. Expected completion by Visionar A.B. post project at month 9.
WP9	17. Report on the clinical, regulatory & commercial requirements for systems to culture expand patient-own adult stem cells	30	3	2	Report	Restricted	Completed on schedule
	18. Standardized culture conditions to expand adult human stem cells, and assays to characterize these cells	30	12	2	Demo	Restricted	Completed on schedule
	19. Proof-of-principle of a system that can comply with the requirements set in task 1 and can be used to expand adult stem cells.	30	12	2	Demo	Restricted	Completed on schedule
	20. Cost-analysis to commercialise the optimised cell expansion method	30	3	2	Report	Restricted	Completed on schedule

Management Deliverables:

WP No.	Deliverable No. & Deliverable Name.	Delivery Date	Est. Person Months	Lead Participant	Nature	Dissemination level	Status
WP0	21. Consortium agreement signed by all partners.	0	0.1	1	Report	Confidential	Completed on schedule
	22. Contracts signed by all partners.	0	0.1	1	Other	Public	Completed on schedule
	23. Updated patent and literature search at start of project	1	0.1	1	Report	Public	Completed on schedule
	24. Updated analysis of competitors' products at start of project	1	0.1	1	Report	Public	Completed on schedule
	25. Optional Project presentation	1	0.1	1	Other	Public	Completed on schedule
	26. six monthly progress reports evaluating extent to which objectives and milestones have been met, and refining future goals/time lines	6, 12, 18, 24, 30	1	1	Report	Restricted	Reports Completed at months 6, 12 and 30
	27. Licenses for *in vivo* trials	11 & 22	0.1	8	Other	Confidential	Completed on schedule
	28. IP management reports	12 & 30	0.5	1	Report	Confidential	Completed on schedule
	29. Filed patent applications	Variable	Var.	Var	Other	Public	Four patents filed
	30. Analysis of product development and commercialization beyond the CRAFT project time line.	12 & 30	1	1	Report	Restricted	Completed on schedule
	31. Final report including cost analysis of products, evaluation of	30	0.1	1	Report	Public	Completed on schedule

	project, and position with regard to competitors at the end of the project.						
	32. Signed audit certificate from each partner.	30	0.1	1	Other	Public	Completed on schedule
	33. SilkBone Project Dissemination Plan	12 & 30	1	1	Report	Public	Completed on schedule

In total, 30 of 33 Project deliverables were completed within the timeframe of the project, with one being modified (deliverable 5), one being delayed (deliverable 16), and two deliverables being abandoned (deliverables 9 and 14). The modified deliverable 5 related to a change in project strategy and the decision not to include the fibre component in the final iteration bone substitute material with the result that the report was modified to one on the effects of cross linking components on the mechanical strengths of fibres produced on WP1. The delay to deliverable 16 is due to the longer than anticipated implantation trials required by the ISO standards to establish long term safety of the SilkBone Bone Substitute Material, and a report is expected by the end of 2009. The decision not to incorporate a source of recombinant human Bone Morphogenetic Protein (rhBMP) in the final iteration Bone Substitute Material meant that deliverables 9 and 14 relating to incorporation of rhBMP and measurement of its release were consequently abandoned.

Dissemination and Use

The goal of the SilkBone project, being a FP-6 CRAFT project focussed on development of high value intellectual property for Europe's SME's, has primarily been to maximise commercial outcomes. Towards this end, work on the SilkBone project has directly contributed to developments for the SME Partners on a wide range of collateral technologies in addition to the core objective of developing the SilkBone™ BSM. These include:

- A biocompatibility assay for trialling implantable biomaterials *in vitro.*
- A novel source of recombinant human Bone Morphogenetic Protein (rhBMP).
- An ELISA kit for *in vitro* measurement of the release of rhBMP's.
- A stem cell bioreactor technology platform, with guidelines on regulatory aspects of stem cell technologies and a feasibility cost/benefit analysis.
- A novel implantable cartilage repair material.
- A novel implantable wound healing material.
- A novel implantable nerve regeneration device.
- A novel implantable wound closure device.
- A catheter introduced prototype heartvalve.
- A prototype orthopaedic soft tissue anchor.
- A prototype absorbable bone fixation plate.

Two of these technologies, the cartilage repair technology and the SilkBone™ BSM will be taken forward by a newly incorporated spin out company, Orthox Ltd. (company registered in England and Wales with Company number 6606448) which has been formed to commercialise the orthopaedic biomaterial outputs of the SilkBone project. Orthox is currently actively seeking equity finance to build the company and take the SilkBone™ BSM, silk fibroin cartilage repair materials and associated orthopaedic devices to the market place. An executive summary of Orthox is provided in appendix 1 of this report and a full business plan is available.

Publishable results of the SilkBone project

The SilkBone project has not only delivered commercial outputs, though the publication of many of the projects results has been delayed to allow the filing of intellectual property. However academic articles arising from the results of the project, and several articles of general or industrial interest have been published:

Holland, C.A., Terry, A.E., Porter, D. & Vollrath, F. Comparing the rheology of native spider and silkworm spinning dope. *Nature Materials* **5**, 870-874 (2006).

Swinerd, V.M., Gheysens, T., Collins, A., Skaer, N. J. V., and Mann, S. (2007) Silk Inverse Opals from Template-directed Beta-sheet Transformation of Regenerated Silk Fibroin. Soft Matter 3(11), 1377 – 1381

Andrew M. Collins[1], Nick J.V. Skaer[2], Tom Gheysens[2], David Knight[2], Caroline Bertram[3], Trudy Roach[3], Richard Oreffo[3], Sonja Von-Aulock[4], Teodora Baris[4], John Skinner[5], Stephen Mann. Bone-like resorbable silk-based scaffolds for load-bearing osteoregenerative applications. *Submitted to Nature Materials June 2008*

Cheng C, Shao Z, Vollrath F (2008) Silk Fibroin regulating the crystallization process of calcium carbonate , Advanced Functional Materials. *(in press)*

Holland, C, Vollrath F (2008) Biomimetic principles of spider silk for high performance fibres. Chapter 7 in *Biologically Inspired Textiles* eds. M.S. Ellison, A. G. Abbott. Woodhead Publishing Ltd. Cambridge. *(in press)*

Yanling Xiao, Victor Peperzak, Linda van Rijn, Jannie Borst and Joost D. De Bruijn Dexamethasone treatment during the expansion phase maintains stemness of Bone Marrow mesenchymal stem cells. (*submitted)*

Liu Y, Shao Z, Vollrath F (2008) Elasticity of spider silks. Biomacromol. *(in press)*

Porter D, Vollrath F (2008) Silk as Biomimetic Model for Polymer Design. Advanced Materials. *(in press)*

Vollrath F, Dicko C, Porter D (2008) Silk. Chapter 9 in 'Handbook of Fibre Structure' (eds S. Eichhorn, M. Jaffe, T. Kikutani). *(in press)*

'Weaving Webs of Wonder' in Oxford Today, Hilary Edition 2008

'Silky Feeling' in Materials World Magazine, August 2007

Publications: two abstracts were submitted for the TERMIS-EU conference and both were selected as oral presentation (TERMIS-EU 8-11/Oct/2006 Rotterdam).

Abstract 118 (TERMIS-EU 2006): Xiao et al. Dexamethasone treatment during the expansion phase improves plasticity of mesenchymal stem cells from human bone marrow.

Abstract 182 (TERMIS-EU 2006): Van Dijkhuizen-Radersma et al. Expansion of multipotent mesenchymal stem cells using microcarriers.

Medical Device Developments, August 2008 Vol.2

Natures 100 Best 'World Changing Innovations Inspired by Nature' Planned release, October 2008

Two filed patent applications have published in 2007

Appendix 1: Executive Summary, Orthox Ltd.

Business Plan for Orthox Limited

Orthox: Regenerating Strength

February 2008

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1. Executive Summary

Orthox Ltd. is a new orthopaedic medical device company being spun out from Oxford Biomaterials Ltd. Orthox will develop cartilage and bone repair products using tissue regenerating scaffolds formed from a ground breaking, absorbable, silk protein polymer.

- **Revolutionary absorbable technology for regenerative tissue repair**

  The Company's products are based on a patented biomaterial technology platform registered under the trademark Spidrex®. Orthox believes Spidrex® is the first absorbable, regenerative tissue technology capable of withstanding the high loads exerted on implants in human joints.

- **Multi billion dollar, underdeveloped, opportunity**

  The orthopaedic biomaterials market is experiencing rapid, sustained growth due to the aging but increasingly active population. Within this sector, Orthox has targeted load bearing applications in which competitive products do not exist or are failing to meet clinical needs.

- **Founded on validated, visionary science**

  Spidrex® has been developed from concept by Professors Knight and Vollrath of Oxford University through their company, Oxford Biomaterials Ltd. Spidrex® has already attracted significant equity finance for medical device developments in wound closure and neural repair.

- **Delivered through a cost effective, commercial process**

  Spidrex® scaffolds are manufactured using a cheap, reliable and scaleable production process using only commercially sourced, regulatory approved, raw materials.

- **Potential for extensive product pipeline diversification**

  Orthox will be licensed to develop Spidrex® technology in all cartilage and bone applications permitting extensive pipeline product diversification throughout the orthopaedic repair sector.

- **Experienced and dynamic management Team**

  Orthox has assembled an ambitious management team that integrates clinical expertise, industry experience, regulatory know-how and scientific excellence allied to a development team that have progressed the technology over the previous three years.

- **Development collaboration with major industry partner**

  Orthox has concluded a development collaboration with a major international medical device company which will trial Spidrex® Cartilage in a large animal model during 2008.

- **Seeking £2 million to gain clinical validation**

  Orthox is now raising £2 million to gain CE marks and clinical validation for its lead products.

2. Company Background

2.1 Origin

Orthox Ltd. is a new spin out company from Oxford Biomaterials Ltd. (www.oxfordbiomaterials.com), which will develop and commercialise silk-based tissue repair products in the field of orthopaedic medicine. Oxford Biomaterials have developed Spidrex®: a proprietary silk technology platform and have already spun out three equity financed ventures to commercialise their products. Two of these, Neurotex Ltd. (www.neurotex.co.uk), and Suturox Ltd., are commercialising Spidrex® nerve repair and wound closure devices, respectively. The third, Spin'tec Engineering Gmbh (www.spintec-engineering.de), is developing cardiovascular devices and biomimetic spinning technology.

2.2 Mission

Orthox will commercialise Spidrex® bone graft and cartilage repair products. Spidrex® Bone has been developed through a €2.4m E.U. consortium from which all biomaterials IP will vest in Orthox. Spidrex® Bone will exploit an identified niche in this $750m market for an absorbable, load bearing, bone graft material. Spidrex® Cartilage has been developed in-house and is being targeted at repair of the meniscal cartilage in the knee which is currently an unsolved medical problem. Spidrex® Cartilage can also be developed for the multi billion dollar articular cartilage and spinal disc repair markets.

2.3 Competitive Edge

Key to the Orthox cartilage and bone products is their great strength combined with slow absorbability, which is conferred by Oxford Biomaterial's proprietary Spidrex® silks. Absorbable devices are highly desirable as they are slowly replaced by regenerating tissue, which obviates the need for expensive and painful follow-up operations to remove defective or worn out devices. However, current absorbable orthopaedic products lack the required strength to adequately compensate for the damaged or missing tissue meaning they are of extremely limited use to surgeons. Orthox products have demonstrated highly promising results in animal trials and, further, support growth of human cartilage and bone cells.

2.4 Vision

Orthox is in possession of sector leading, patented, technology which has demonstrated great potential when evaluated in bone and cartilage models, resulting in a development partnership with a leading international medical device company. Orthox has assembled an ambitious and experienced management team and has access to state of the art research and clinical facilities in both the UK and Continental Europe. The Orthox vision is, over the next three years, to progress the technology through large animal and clinical trials, achieving CE marked cartilage and bone graft products. Trade sale will be a potential exit at this point, or follow-on funding may be sought in 2011 to upscale manufacturing and marketing and establish Orthox as a leading orthopaedic device company within the next five years.