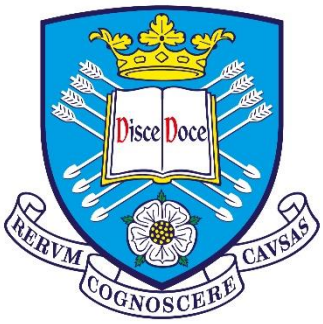

The University Of Sheffield.

DEPARTMENT OF

MATERIALS SCIENCE AND ENGINEERING

Development of Osteoinductive Coatings for Spinal Implants (Fusion Cages)

1st Year Confirmation Report

From 3rd September 2018 – 11th July 2019

Jose Rui Oliveira Rodrigues

Supervised by Dr. Frederik Claeyssens and Dr. Gwendolen Reilly

1. Introduction and Aims

As populations grow old, the global impact of musculoskeletal disorders has been increasing, even in developing countries. In fact, these disorders have a disability impact of 21.3% of total years lived with disability (YLD), ranking second only to mental health and behavioural problems [1]. In terms of total global disability adjusted life years (DALY) musculoskeletal conditions rank fourth in global burden, affecting 6.7% patients [1]. Yet, if we look at each disorder on its own, lower back pain appears as the leading cause of YLD, and is sixth in terms of DALY [1-3]. From a public health level, disability adds to the already high economic and social impact of lower back pain and at a patient level, it significantly affects one's socio-economic status and quality of life [2, 3]. As such, it is of great importance to prevent or treat lower back pain in a timely manner

As a treatment for back pain, surgery is generally the last option given to patients, recommended only to patients that show intervertebral disk herniation or degeneration [4]. For these cases, spinal fusion is one common procedure [4-7]. It consists in the fusion of adjacent vertebra by placing an implant named "spinal fusion cage" between them [8]. While this procedure has lower rates of postoperative complications than other similar spinal surgeries, they are still high enough for surgery to not be recommended at early stages of detection of back pain [4, 8]. In parallel, research on improved fusion cages continues to this day, to reduce the rates of postoperative complications. The goal is to improve implant osteointegration and fusion rates, minimize subsidence, and, consequently, improve success rates [5, 6, 9].

The aim of this project will be to develop a novel hydroxyapatite-based coating for spinal fusion cages. Coatings have a proven record of improving the integration or orthopaedic implants including fusion cages [5, 9-11] Hydroxyapatite (HA) is a material of great interest, being the main mineral in bone, and having osteoconductive and bone-binding properties [12-14]. To overcome the quality drawbacks shown by the standard HA-coating techniques [15-17], sol-gel chemistry will be used. This is a low-cost technique that is able to produce high-quality coatings [18, 19]. This project will also study the development of HA with Mg and Sr substitutions, which more closely follow the composition of bone HA and have enhanced bioactivity [14]. Mesenchymal stem cells are the precursors to bone cells and one of the first cells types to encounter and implant surface after surgery. Therefore the response of mesenchymal stem cells to an implant is an important factor in determining whether bone integration will occur [20, 21].

The project has four main objectives:

I. Development of a new serum-free protocol for expansion and bone differentiation of Mesenchymal Stem Cells;
II. Development of HA-based osteoinductive coatings;
III. Development of new substituted HA formulations;
IV. Coating of spinal fusion cages.

2. Work Complete to Date

2.1. Materials and Methods

Human Mesenchymal Stem Cells (hMSCs) at different passages (P3 and P4) were cultured in different culture media. The details of each media are presented in table 1. Media BM1 and BM2 contained Foetal Bovine Serum (FBS) from two different sources. FBS is the standard supplement for cell culture media. HMS is a specialized medium for hMSC culture containing human serum. CD1 and CD2 are commercially available serum-free alternatives for hMSC culture. Three substrate conditions were also tested, based on the recommendations from the serum-free media providers and the laboratory standards. These were no-coating, gelatine coating (GEL, 0.1% w/v) and bovine fibronectin coatings (FIB, 10 µg/ml). The hMSCs were cultured with a cell density of 13333.33 cells/cm^2, and the media changed every 2-3 days until cells reached ≥ 80% confluence. Cell expansion was evaluated through light microscopy and cell metabolic activity was measured through resazurin reduction assay.

***Table 1** - Media composition*

MEDIA	COMPOSITION	CLASSIFICATION
BM1	α-MEM (Lonza, Cat no. 12-169F) 10% FBS (Labtech, Lot 40811)	SC
BM2	α-MEM (Lonza, Cat no. 12-169F) 10% FBS (Gibco, Lot 08F7675K)	SC
CD1	StemMACS MSC Expansion Media Kit XF, human (Miltenyi Biotec, Cat no. 130-104-182)	SF, XF
CD2	Mesenchymal Stem Cell Growth Medium DXF (PromoCell, Cat no. C-28019)	SF, XF, CD*
HSM	**Human Mesenchymal-XF Expansion Medium** (Merck, Cat no. SCM045)	SC, XF

SC – Serum Containing; SF – Serum-free; XF – Xeno-free; CD – Chemically Defined.
* While suppliers claim the media as “defined”, it might not be chemically defined.

2.2. Results and Discussion

While FBS is the most used supplement for culture media, its undefined nature can lead to several drawbacks. For this project, lot-to-lot variability is concerning, since different FBS can lead to cultures with different results [22, 23]. The results obtained seem to corroborate these claims. While not statistically significant, there is a visible difference between cultures with BM1 or BM2. In this case, BM2 presents itself as a slightly better alternative (figure 1). Other works have shown more disparate results when comparing FBS from different sources [24].

The obtained results also show why specialized media might be a necessity for MSC culture. Both CD1 and HSM were significantly better for MSC expansion. There was a significant increase in metabolic activity when using these media (figure 1). Cultures using CD1 and HSM also reached 80% confluence at a faster rate (figure 2). This goes in line with the literature, which shows that media specialized for MSC culture support higher growth rates [23]. For HSM, which contains human serum, it also shows the benefits of supplementing media with human serum for cell expansion against FBS. However, the use of human serum still shares some of the drawbacks FBS has, such as lot-to-lot variability and risk of infection [23, 25]. The fact that a serum-free media such as CD1 is able to reach similar performance to HSM shows that serum-free media can be viable alternatives to serum-containing media.

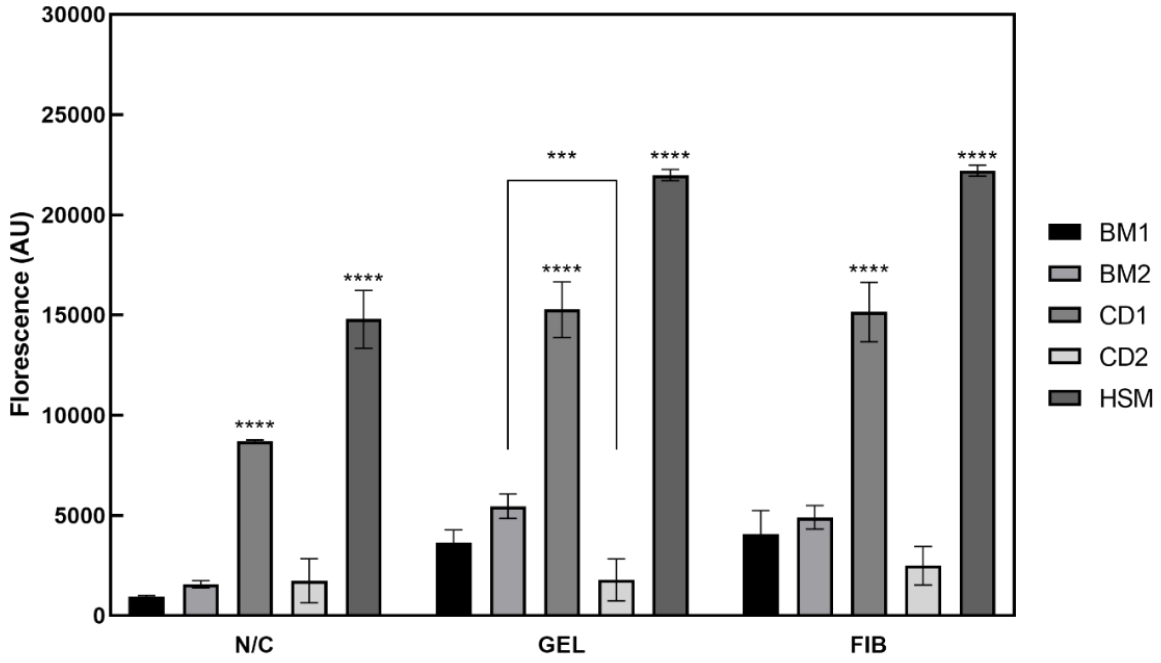

*Figure 1 - Mean results from resazurin reduction assay for each media cultured in different substrates on day 7. *** and **** represent statistically different results (*** $p < 0.001$; **** $p < 0.0001$).*

The outlier is CD2, which is also a specialized media for MSC expansion, yet it showed results similar to BM1 and BM2, even in FIB coated substrates as recommended by its supplier. In fact, contrary to all other media, where the use of a coated substrate was beneficial, using a coated substrate did not affect CD2 cultures at all. This could mean that the formulation of CD2 is not well optimized. However, it could also mean that the specific finite MSC cell line does not react well to this specific media. Other works showed that media specialized for a specific MSC line/primary source is needed for better MSC cultures. Some research groups prefer to use their own in-house formulations for these reasons [23, 26]. It is possible that CD2 simply is not well optimized for these particular cells and not for MSCs in general.

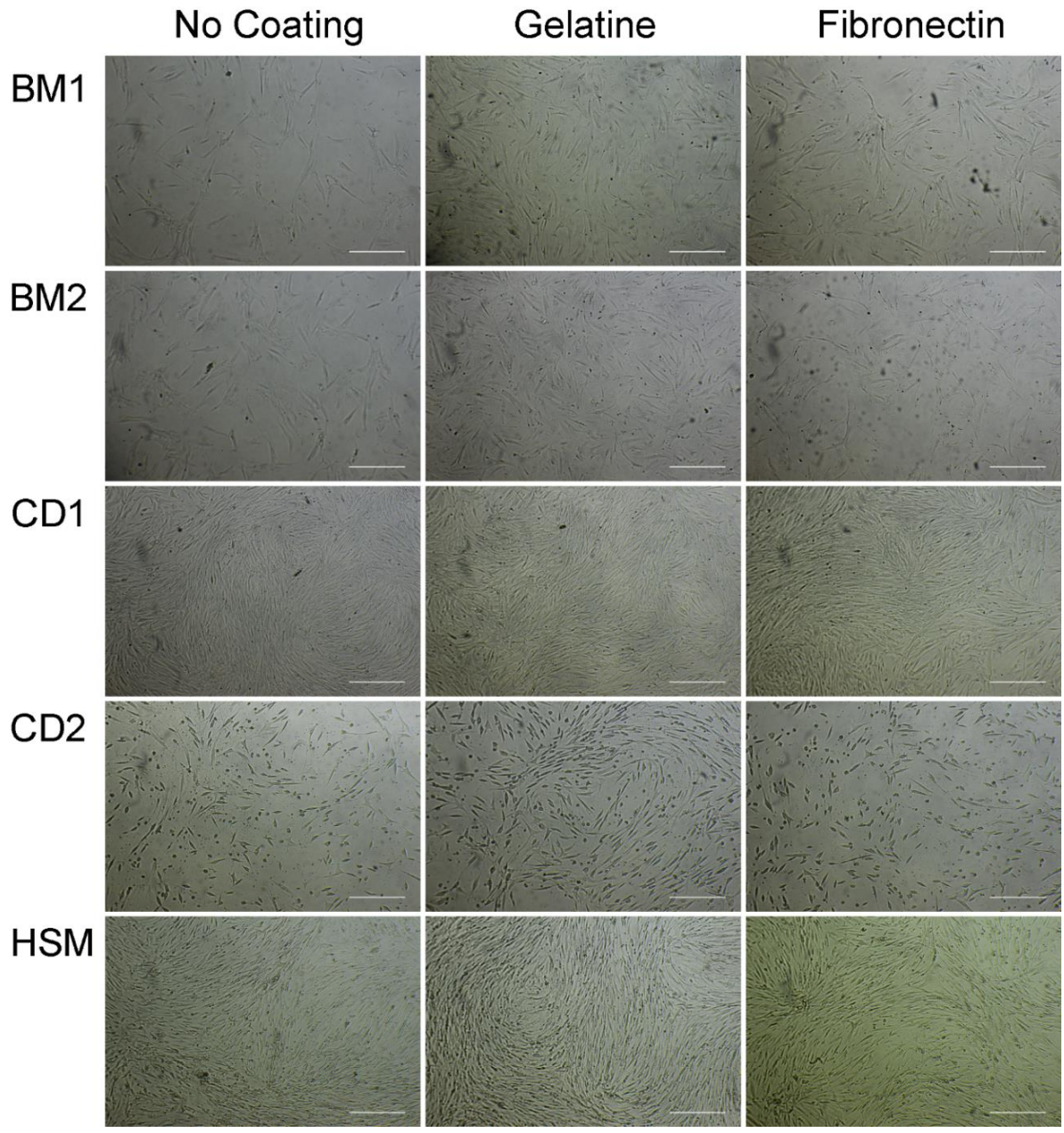

Figure 2 - *Cell expansion in different media and different substrate at day 7. Scale bar = 500 µm.*

3. Planned Future Work

I. **Development of a new serum-free protocol for expansion and bone differentiation of Mesenchymal Stem Cells:**

The cell culture experiments will be repeated with an immortalized MSC line. Immortalized cells do not change characteristics with increased culture, and as such are better suited for repeated experiments over long periods. These new results will help to confirm the optimal serum-free conditions for MSC expansion.

The next step will be to study the best culture conditions for bone differentiation of both finite and immortalized MSCs. This will be studied using the same media used for cell expansion, with or without proper supplements.

After obtaining the best results for MSC expansion and differentiation, a protocol will be developed to be used for any cell culture experiments necessary for SPINNER and other future projects.

II. **Development of HA-based osteoinductive coatings**

The coatings will be developed using the sol-gel chemistry technique. The coatings will explore a TiO_2/HA composite formulation to improve adhesion to substrates without losing the osteoinductive properties of HA. The coatings will be testes in both Titanium and PEEK substrates, as these are the standard materials used to produce spinal fusion cages.

A Design of Experiments (DoE) approach will be used to study which factors most affect the development of the coatings, and which are the optimal conditions for the process. DoE allows to estimate these factors and conditions with a smaller number of experiments than testing each condition on its own. It also allows for studying the interaction between factors and their effect on the properties of the coating.

III. **Development of new substituted HA formulations**

This objective is part of an industrial secondment with Finceramica in Faenza, Italy. A DoE approach will also be used to study the development of new formulations for substituted hydroxyapatite. The goal is to develop formulations that have either increased osteoinductive or antibacterial properties.

IV. **Coating of spinal fusion cages**

This objective is part of an industrial secondment with Aesculap in Tuttlingen, Germany. The coatings developed during objective II, allied with the HA developed during objective III, will be tested in spinal fusion cages. The newly coated cages will then be evaluated

using industry standards and compared cages coated with standard techniques. The new coatings will be optimized according to said results.

4. References

[1] A.M. Briggs, M.J. Cross, D.G. Hoy, L. Sanchez-Riera, F.M. Blyth, A.D. Woolf, L. March, Musculoskeletal Health Conditions Represent a Global Threat to Healthy Aging: A Report for the 2015 World Health Organization World Report on Ageing and Health, Gerontologist 56 (2016) S243-S255.
[2] H. Lee, M. Hubscher, G.L. Moseley, S.J. Kamper, A.C. Traeger, G. Mansell, J.H. McAuley, How does pain lead to disability? A systematic review and meta-analysis of mediation studies in people with back and neck pain, Pain 156(6) (2015) 988-997.
[3] C. Maher, M. Underwood, R. Buchbinder, Non-specific low back pain, Lancet 389(10070) (2017) 736-747.
[4] O. Airaksinen, J.I. Brox, C. Cedraschi, J. Hildebrandt, J. Klaber-Moffett, F. Kovacs, A.F. Mannion, S. Reis, J.B. Staal, H. Ursin, G. Zanoli, C.B.W.G.o.G.f.C.L.B. Pain, Chapter 4. European guidelines for the management of chronic nonspecific low back pain, European spine journal : official publication of the European Spine Society, the European Spinal Deformity Society, and the European Section of the Cervical Spine Research Society 15 Suppl 2(Suppl 2) (2006) S192-S300.
[5] S. Jain, A.E.M. Eltorai, R. Ruttiman, A.H. Daniels, Advances in Spinal Interbody Cages, Orthopaedic Surgery 8(3) (2016) 278-284.
[6] K. Phan, R.J. Mobbs, Evolution of Design of Interbody Cages for Anterior Lumbar Interbody Fusion, Orthopaedic Surgery 8(3) (2016) 270-277.
[7] R. Kersten, S.M. van Gaalen, A. de Gast, F.C. Oner, Polyetheretherketone (PEEK) cages in cervical applications: a systematic review, Spine Journal 15(6) (2015) 1446-1460.
[8] A.J. Talia, M.L. Wong, H.C. Lau, A.H. Kaye, Comparison of the different surgical approaches for lumbar interbody fusion, Journal of Clinical Neuroscience 22(2) (2015) 243-251.
[9] P.J. Rao, M.H. Pelletier, W.R. Walsh, R.J. Mobbs, Spine Interbody Implants: Material Selection and Modification, Functionalization and Bioactivation of Surfaces to Improve Osseointegration, Orthopaedic Surgery 6(2) (2014) 81-89.
[10] B.J. McEntire, B.S. Bal, M.N. Rahaman, J. Chevalier, G. Pezzotti, Ceramics and ceramic coatings in orthopaedics, Journal of the European Ceramic Society 35(16) (2015) 4327-4369.
[11] R. Junker, A. Dimakis, M. Thoneick, J.A. Jansen, Effects of implant surface coatings and composition on bone integration: a systematic review, Clinical Oral Implants Research 20 (2009) 185-206.
[12] G.F. de Grado, L. Keller, Y. Idoux-Gillet, Q. Wagner, A.M. Musset, N. Benkirane-Jessel, F. Bornert, D. Offner, Bone substitutes: a review of their characteristics, clinical use, and perspectives for large bone defects management, Journal of Tissue Engineering 9 (2018) 18.
[13] W. Suchanek, M. Yoshimura, Processing and properties of hydroxyapatite-based biomaterials for use as hard tissue replacement implants, Journal of Materials Research 13(1) (1998) 94-117.
[14] K. Lin, J. Chang, 1 - Structure and properties of hydroxyapatite for biomedical applications, in: M. Mucalo (Ed.), Hydroxyapatite (Hap) for Biomedical Applications, Woodhead Publishing2015, pp. 3-19.
[15] R.I.M. Asri, W.S.W. Harun, M.A. Hassan, S.A.C. Ghani, Z. Buyong, A review of hydroxyapatite-based coating techniques: Sol-gel and electrochemical depositions on biocompatible metals, Journal of the Mechanical Behavior of Biomedical Materials 57 (2016) 95-108.
[16] S.R. Paital, N.B. Dahotre, Calcium phosphate coatings for bio-implant applications: Materials, performance factors, and methodologies, Materials Science & Engineering R-Reports 66(1-3) (2009) 1-70.
[17] R. Narayanan, S.K. Seshadri, T.Y. Kwon, K.H. Kim, Calcium phosphate-based coatings on titanium and its alloys, Journal of Biomedical Materials Research Part B-Applied Biomaterials 85B(1) (2008) 279-299.
[18] A.E. Danks, S.R. Hall, Z. Schnepp, The evolution of 'sol-gel' chemistry as a technique for materials synthesis, Materials Horizons 3(2) (2016) 91-112.
[19] U. Schubert, Chemistry and Fundamentals of the Sol-Gel Process, The Sol-Gel Handbook (2015).
[20] R.J. Miron, Y.F. Zhang, Osteoinduction: A Review of Old Concepts with New Standards, Journal of Dental Research 91(8) (2012) 736-744.
[21] M.C. Vemuri, L.G. Chase, M.S. Rao, Mesenchymal Stem Cell Assays and Applications, in: M. Vemuri, L.G. Chase, M.S. Rao (Eds.), Mesenchymal Stem Cell Assays and Applications, Humana Press, Totowa, NJ, 2011, pp. 3-8.
[22] N. McGillicuddy, P. Floris, S. Albrecht, J. Bones, Examining the sources of variability in cell culture media used for biopharmaceutical production, Biotechnology Letters 40(1) (2018) 5-21.
[23] S. Gottipamula, M.S. Muttigi, U. Kolkundkar, R.N. Seetharam, Serum-free media for the production of human mesenchymal stromal cells: a review, Cell Proliferation 46(6) (2013) 608-627.
[24] J. Melke, Environmental stimuli for controlled bone tissue engineering applications, Eindhoven University of Technology, 2019, p. 172.
[25] S. Shanbhag, A. Stavropoulos, S. Suliman, T. Hervig, K. Mustafa, Efficacy of Humanized Mesenchymal Stem Cell Cultures for Bone Tissue Engineering: A Systematic Review with a Focus on Platelet Derivatives, Tissue Engineering Part B-Reviews 23(6) (2017) 552-569.
[26] O. Karnieli, O.M. Friedner, J.G. Allickson, N. Zhang, S. Jung, D. Fiorentini, E. Abraham, S.S. Eaker, T.K. Yong, A. Chan, S. Griffiths, A.K. When, S. Oh, A consensus introduction to serum replacements and serum-free media for cellular therapies, Cytotherapy 19(2) (2017) 155-169.