Chondrocytes, Mesenchymal stem cells and Co-culture

Contents *Page*

2.0 Written proposal

2.1 Project Title

To evaluate the proliferation and differentiation capacity co-culture of equine umbilical cord blood and bone marrow derived mesenchymal stem cells with nasal and articular chondrocytes.

2.2 Project summary

Stem cell development is a new and exciting field, especially regarding the treatment of joint disease in the horse. There have been numerous peer reviewed studies published detailing the utilization of mesenchymal stem cells (MSCs) from numerous biological sites in musculoskeletal disease in horses and humans. More recently favourable results have been obtained from the co-culture of MSCs with chondrocytes (Chs) for cartilage repair application in humans as well as animal models. The cell sources produce a synergistic effect on proliferation, gene expression and production of an extracellular matrix like that of native cartilage.

Allogenic MSCs utilization is an ongoing research area. The immune properties of stem cells suggests that allogeneic cell sources are immune privileged, compared to adult cells and have immune modulatory properties, thus they may be a viable option for treatment going forward. Work in this area is aimed at defining the optimal characteristics and developing a readily available cell source with ideal properties. Allogeneic sources of mature differentiated cells types like Chs are more problematic due to donor-recipient reaction. The procedure to collect autologous articular Chs involves a general anaesthetic and an invasive procedure to obtain articular cartilage, which makes this highly impractical, ethically and financially. Allogenic tissue engineering with Chs requires further research to allow further utilization of these non-immune privileged cells to reduce the number of invasive procedures and general anaesthetics required.

There is a large gap in present knowledge and development of protocols for the obtainment of nasal Chs in horses and their co-culturing with MSCs (both autogenic and allogenic) for tissue engineering application. The use of nasal Chs

would reduce the requirement for general anaesthetics. However, there is also a requirement to evaluate if nasal Chs contain equivalent chondrogenic properties as those obtained from articular cartilage for co-culture application for cartilage tissue engineering.

The objective of this literature review is to summarise the current published work on co-culture of MSCs and chondrocytes, with special attention to different Ch sources (i.e. articular vs. nasal). To evaluate the co-culture abilities of allogeneic equine umbilical cord blood derived and autogenic bone-marrow derived MSCs with nasal and articular Chs with further side evaluation of nasal Chs vs articular cartilage performance.

3.0 Literature Review

3.1 Articular cartilage

Cartilage is a smooth elastic tissue that encapsulates and protects the proximal and distal articulations of joints (Sjaastad, Hove, & Sand, 2016). Cartilage is composed of specialized chondrocyte cells that produce large amounts of collagenous extracellular matrix and amorphous gel-like substances, which have high concentrations of proteoglycans, elastin fibers and can store large amounts of water (Akers & Denbow, 2013). Cartilage is avasucular and aneural in which nutrition is therefore supplied to the chondrocytes via diffusion (Akers & Denbow, 2013). When the cartilage is compressed, it expels fluid and absorbs fluid when expanded, thus supplying nutrients to the chondrocytes and removal of waste products. Cartilage can be further divided into three categories; elastic, hyaline and fibrocartilage, which are differentiated by their collagen and proteoglycan concentrations (Sjaastad et al., 2016).

As a result of the avascular and aneural characteristics, cartilage has a slow turn-over of extracellular matrix and therefore very slowly/ does not repair (Akers & Denbow, 2013)Refer to figure 6 for a depiction of the slow process.

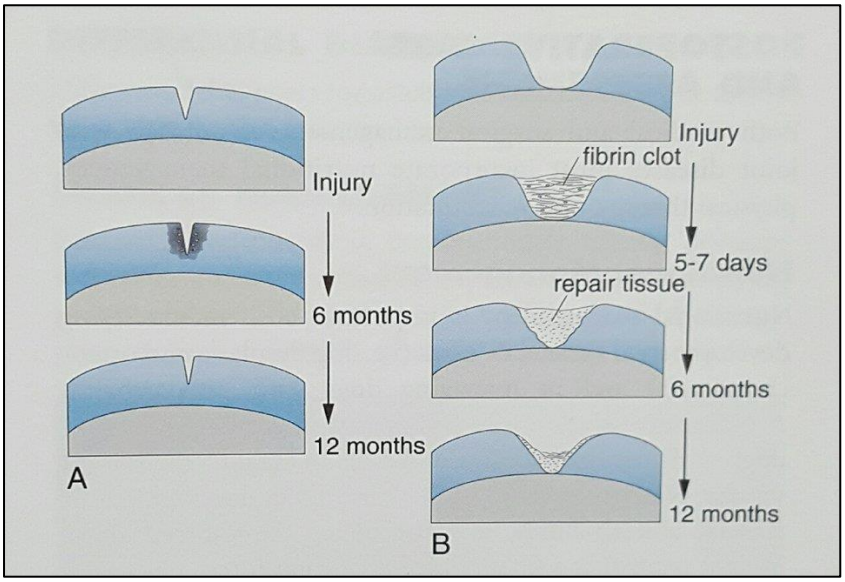

Figure 1a: The healing phases of (a) superficial and (B) injuries of the articular cartilage. Picture obtained from Fossum et al., 2013.

Hyaline cartilage is the most prevalent and important tissue encapsulating bones, forming the articular surface within the joint capsule (Akers & Denbow, 2013).It has a high presence of collagen fibers, thus provides a strong, less elastic cover on the mobile surface (Sjaastad et al., 2016).In a histological slide of the lamb femur under the microscope, multiple chondrocytes can be identified as a blunt angular cells within groups amongst the homogenous matrix. Refer to figure 1a (see appendix A) for further detail. They consist of clear translucent protoplasm with two

or more round nuclei. The chondrocytes are contained within cartilage lacunae. (The gap around the cell is artificial, caused by the staining process) (Fossum, Duprey, & Huff, 2013).

Articular cartilage can be further divided into four zones; Subchondral bone (Trabeculae bone), calcified cartilage, deep zone, transitional bone and the superficial zone. Refer to the annotated drawing, figure 1b, see appendix B. The superficial layer is the shiny articulating surface in contact with the neighbouring bone. The transitional zone is composed of spherical chondrocytes, collagen fibrils and extracellular matrix. The deep zone is made up of perpendicular columns of smaller chondrocytes. The deepest cartilage zone is the calcified cartilage zone which anchors the cartilage to the subchondral bone(Fossum et al., 2013)

3.2 Horses anatomically superior and functionally similar to humans

Laboratory rodents have been extensively utilized for the understanding of cellular therapy for musculoskeletal disease (Vo et al., 2013). However, rodents are anatomically inferior to the equine model due to their minute cartilage thickness, joint size and their minimal joint forces(Chu, Szczodry, & Bruno, 2010). The stifle joint of a horse closely approximates the human knee compared to other animal models(McIlwraith, Fortier, Frisbie, & Nixon, 2011). Full thickness histological measurements of cadaveric specimens were obtained from the human, horse, goat, dog, sheep, and rabbit. These measurements were taken from five locations including non-calcified and calcified cartilage layers, as well as the subchondral bone plate. Over the five locations the articular cartilage thickness averaged; 2.2 to 2.5 mm for humans, 0.3 mm for rabbits, 0.4 to 0.5 mm for sheep, 0.6 to 1.3 mm for dog, 0.7 to 1.5 mm for goat, and 1.5 to 2.0 mm for horse(McIlwraith et al., 2011).

In recent literature review, Colbath et al literature highlights that experimental equine studies over rodent studies, particularly the fact that equine studies offer the ability to produce a controlled exercise regime to provide a standardize postsurgical intervention activity log, and thus accurate prognostic results in regard to healing and reinjury. The horse therefore provides an excellent preclinical model by achieving superior clinical relevance for transitioning promising research from small animal models to human clinical trials (Colbath et al., 2017).

Horses' naturally acquire articular cartilage trauma inducing disease processes, including: osteoarthritis (OA), osteochondritis dissecans (OCD), meniscal and other soft tissue injury, ultimately providing researchers opportunities for both experimental and clinical studies.

One of the limiting factors in equine research is the increased cost associated with logistically handling equine surgeries compared to that of the rodent studies(Chu et al., 2010). The number of animals is significantly reduced because of this associated cost as well, which potentially could result in lack of statistical significance in results; therefore careful power calculations are needed when planning a study(Chu et al., 2010). The horse overall provides a very unique preclinical situation due to the ability to utilize imaging modalities and an exercise regime similar to that of humans. Horses provide a platform for safe testing and efficacy of cellular therapies thus enabling easy translation as they routinely experience similar conditions to that of human musculoskeletal disease(Colbath et al., 2017).

Cellular therapy in tissue engineering research for musculoskeletal disease in humans has developed phenomenally in recent years. However, in the human research there are unanswered question and significantly more in the equine world regarding the optimal ratios for MSC and Ch application. The equine model is incredibly useful in identifying the optimal cell ratio and scaffold system to produce the most effective results(Colbath et al., 2017).

Controlled experimental trials are essential to ensure the strength of feasibility of cartilage repair constructs, as well as answering remaining questions on activity of MSC, chs and their interaction within the selected scaffolding systems(Colbath et al., 2017). The cartilage of the horse is anatomically similar to the human, but subjected to higher loads *in vivo;* therefore, the equine model will play an important role in advancing the field of musculoskeletal of regenerative medicine(Chu et al., 2010).

3.3 Previous Research for chondrocytes, mesenchymal stem cells

Many peer reviewed articles have indicated the repair techniques currently utilized for degeneration of articular cartilage do not fully repair damaged articular tissue their full mechanical strength compared to physiological state. The techniques such as abrasion arthroplasty, micro-fracture or meniscal tissue implantation to highlight a few techniques, results in the development of fibrocartilage, which lacks the structural integrity and biochemical properties of hyaline cartilage(Nazempour & Van Wie, 2016).

Chondrocytes, the active cells within cartilage, produce and maintain the cartilaginous matrix (collagen and proteoglycans, mainly aggrecan). Chondrogenesis is the biological process to produce hyaline, fibrious or elastic cartilage. During embryonic development chondrogenesis occurs resulting from condensation and differentiation of mesenchymal cells (Calabrese et al., 2017). The chondrocytes can either remain in a quiescent state to produce articular cartilage or undergo proliferation, terminal differentiation through to chondrocyte hypertrophy and endochondral ossification. Whereby the hypertrophic cartilage is replaced by bone. Many human and equine tissue engineering trials are developed due to the inability of the autologous chondrocytes to lay down new extracellular matrix with the same mechanical properties formed during development (Calabrese et al., 2017).

Cell based and biologic approaches such as autologous articular chondrocyte implantation (ACI) have been developed to better address full thickness cartilage lesions with the goal of creating an improved repair tissue. ACI is commonly used for large full depth subchondral defects. It requires an open surgergical implantation of autologous ex-vivo cultured Chs, which are harvested via biopsy arthroscopically from a prior surgery (Foldager, 2013). This is a highly advanced and expensive procedure which is limited by, obtainment of the articular cartilage, donor site morbity and detrimental effects to the surrounding cartilage. Additionally, it is debatable whether the cartilage obtainment site is truly non-weight bearing. (Nazempour & Van Wie, 2016).

Nazepour et al has highlighted that the procedure to collect autologous articular chondrocytes involves a general anaesthetic and an invasive procedure, which collectively with the other procedures required, makes this highly impractical in humans and ethically and financially challenging in the horse. Nazepour & Van Wie have demonstrated in their research that there is densification of the articular Ch implantation resulting in mechanical stiffening of the subchondral bone, leading to breakdown in repetitive loading situation if only articular Ch implantation is used. It also statistically shows that insufficient concentrations of articular Chs, results in their de-differentiation upon monolayer expansion resulting in hypertrophy, which requires further surgical intervention to correct and thus a major limitation for articular cartilage repair using API (Nazempour & Van Wie, 2016).

Peterson, et al, Brittberg and Lindahl, Viste, et al, and Zaslav, et al have all displayed high success rates for API, even with Zaslav et al's team demonstrating that a success is possible following a failed first attempt. However, Foldager highlights that despite these high success rates and expectations, this articular cartilage is unable to regenerate articular cartilage to a consistent and predicable fashion. In the *in vitro* conditions cell proliferation potential drastically decreases along with de-differentiation and lose of extracellular matrix secretion capabilities (Nazempour & Van Wie, 2016).

Never-the-less, longer follow-up periods are required to truly detain whether ACI is effective before a decision is made as to whether to use this procedure in clinical practice.

In contrast to the articular derived chs, nasal obtained chs display exciting properties (Nazempour & Van Wie, 2016). Nasal derived chs proliferate four times faster than articular chs , secrete twice as many cytokines and exhibit chondrogenic potential independent of donor age (Kafienah et al., 2002). Pelttari et al has demonstrated with their goat articular defect studies that the implantation of autologous non-weight bearing nasal chs leads to the repair of weight bearing articular cartilage within only 6 months. This study also contextualized that autologous non-weight bearing nasal chs displayed superior characteristics to that of autologous articular chs. This work highlights the successfulness and obtainability of a less invasive donor site for tissue engineering applications (Pelttari et al., 2014).

In the field of tissue engineering, much focus has been on MSCs as the optimal cell source. MSCs are undifferentiated plastic adherent cell with a fibroblast like morphology (Berglund, Fortier, Antczak, & Schnabel, 2017). This indicates they can differentiate into a variety of generative cells; bone, cartilage or fat *in vitro* and have a defined set of surface markers which differ between species (Berglund et al., 2017). In the case of cartilage development MSCs lose their pluripotency, proliferate and aggregate at the location of the chondrification. The differentiated chondrogenic cells then go on to synthesize cartilage extracellular matrix to a biomechanical composition and properties of newly formed full thickness hyaline cartilage (H. Zhang et al., 2017). MSCs provide sufficiently larger quantities of cells that are more feasible to obtain via less invasive procedures compare to that of chs harvesting (Nazempour & Van Wie, 2016).

Bone marrow was the first identified utilizable source of MSCs (Nazempour & Van Wie, 2016). This cellular product is limited due to it invasive obtainment in humans, adjacent decrease in MSC concentration and differentiation potential

with increasing age in horses and humans; therefore, alternative biological sites are under investigation to isolate MSCs (Kern, Eichler, Stoeve, Klüter, & Bieback, 2006). Interestingly Zhang, et al literature identifies human bone marrow derived MSCs (hBM-MSCs) as a non-hematopoietic pluripotent cells with the scope of self-renewal and multi-differentiation. hBM-MSCs have immunomodulatory effects on dendritic cells, natural killer cells, t and B lymphocytes. They display suppressive effects on both the innate and humoral immunity by inhibiting the dendritic maturation, natural killer/B-cell activation and T cell proliferation, while still stimulating T cell development(H. Zhang et al., 2017).

Bone marrow derived MSCs account for a highly minute percentage of cells obtained and require expansion before use (Bieback, Kern, Kocaomer, Ferlik, & Bugert, 2008). Although bone marrow derived MSCs show the most chondrogenic potential, the current methods to isolate substantial human autogenic quantities for effective tissue engineering poses a significant ethical dilemma as the obtainment of other MSCs isolates is available (Kern et al., 2006).

One proposed alternative source of MSCs is umbilical cord blood derived MSCs (H. Zhang et al., 2017). Multipotent MSCs are isolated full term without harm to either mother or infant. Harvesting of umbilical cord blood derived MSCs is still an area of much controversy due to public ignorance and requires further protocol establishment and public education (Kern et al., 2006). Umbilical mesenchymal stem cells have twice the amount of population doubling compared to that of bone MSCs and adipose MSCs respectively (Nazempour & Van Wie, 2016). Zhang et al's literature reposts that different sections of human placenta contain stem cells with similar phenotypic, functional and immunomodulatory as Hbm-MSCS. It was also highlighted that the infusion of human umbilical cord derived MSCs (hUC-MSCs) into recipient mice ameliorated acute graft-versus host disease (aGVHD). This highlights the revolutionary potential this treatment application could have for aGVHD caused by allogeneic stem cell transplantation (H. Zhang et al., 2017).
Adipose is also another less invasive alternative biological source to isolate MSCs which possess higher chondrogenic potential to that of BMSCs (Calabrese et al., 2017).

Further understanding of the in vivo differentiation mechanisms of MSCs would allow for precise cell lineage control in the in vitro environment. Bioactive cellular signalling is required for chondrogenic differentiation of MSCs such as; transforming growth factor –b (TGF-b) or Insulin-like growth factor-1 (IGF-1). Primary chondrocytes secrete these bioactive cellular signals, TGF-b and IGF-1, which optimally provides a stage for physiological mediated differentiation of the MSC by Chs producing neo-cartilage that has the potential to resist hypertrophic maturation and calcification.

Allogenic MSCs provide attractive potential, due to their immediate application at the time of the tissue injury or disease diagnosis. Berglund, Fortier Antczak and Schnabel;s literature however highlights that previous trials have identified allogenic MSCs as supposable being immune privileged. However there has been few studies that control for matched or mismatched major histocompatibility complex (MHC) molecule expression (Berglund et al., 2017). The studies that controlled for MHC responses have reported that there was both a humoral and cell-mediated response

(Berglund et al., 2017). For allogenic MSC therapy to be utilized, further investigation into immune responses towards allogenic and autogenic cells are required. Also beneficial will be the effect of these immune responses on the therapeutic outcome of the cells.

The train has now been directed towards developing optimum protocols for co-culturing of MSCs (umbilical and bone marrow derived) with nasal chondrocytes. These techniques have been demonstrated as both obtainable under minimally invasive conditions. Nazempour, et al highlights that there is minimal current research been undertaken in this area and encourages research to broadly investigate cross co-culturing of umbilical blood derived MSCs and bone MSCs with nasal chs.

3.4 Co-Culturing Environments

Chs and MSCs are highly utilized biological cell sources used for cartilage tissue engineering, however as highlighted they both have their associated construction disadvantages when applied in vivo(Zuo et al., 2013). Co-culture provides synergistic effects, reducing the percent of required ch, promote chondrogenic differentiation of MSCs and enhance extracellular matrix (ECM) formation. Co-culture maintains sensitivity to the inducers of the pluripotent differentiations of MSCs. The functional mechanism is achieved through cell-cell interactions mediated by secreted factors; cytokines, growth factors and immunomodulatory cells. Collectively MSC-Ch co-culture requires smaller Ch ratios, abating donor site morbidity while enhancing cartilage repair(Colbath et al., 2017).

Obtainment of optimal MSC:Ch co-culture ratio is key to high quality tissue engieenered cartlage (Zuo et al., 2013). However through Zuo et al's literature different environmental conditions of co-culture resulted in differential optimal ratios. Zuo et al review of Fischer et al found that when human Chs were co-culturedwith human bone marrow derieved MSCs at ratios of 1:1 and 2:1 that there was increased gene expression of Col II. Yang et al, highlights that 63:1 Ch:MSC ratio resulted in chondrogenic development of MSC induced by Chs. In Zuo et al's experiment a 2:1 articular Ch: bone marrow derived MSC ratio was adopted as from previous studies from rabbit co-cultures they had identified this as the optimal ratio of articular Chs to bone marrow derived MSCS(Zuo et al., 2013).

Nazempur, et al literature review highlights that the co-culturing of a 2:1 ratio of bone MSCs with articular Ch results in higher production of GAG and Col II, which theoretically resembles the *in vivo* niche. This ratio induces the molecular signalling of articular chs which stimulates MSC chondrogenesis (development of MSCs into chondrocytes). Due to this stimulation, it can be surmised that reduced numbers of chs are required due to MSCs expansion capabilities, thus reducing articular chs de-differentiation upon their own expansion (less plastic than the MSC, creating fibroblast chs that produce poor extracellular matrix). It can also be noted from Nazempour et al's research that upon MSCs supplementation that smaller articular Ch biopsies are required, significantly reducing donor site morbidity. Nazempour et al's research also brings to light the co-culturing with isolated osteoarthritic articular chs results in chondrogenesis of MSCs even without exogenous supplementation. There is currently minimal research comparing healthy and osteoarthtic articular chs. If reoccurring satisfactory results could be obtained using osteoarthritic articular

chs then the use of existing osteoarthroitic cartilage may become a protocol option if other sites are limited (Nazempour & Van Wie, 2016).

It is apparent from Yang, Lee and Barabino's studies that higher articular chs to MSCs ratios result in maximal production of hypertrophic Collagen X. All of these studies have highlighted the evidently obvious point that a full range of MSC:ACh ratios are required to isolate the optimum ratio, to ensure strength to this potentially life changing work before it can be used for clinical application.

This goal cannot be achieved without characterization by flow cytometry via markers to highlight the chondrogenic, osteogenic and fibrogenic properties of the cells to biomechanically analyse what is occurring and therefore strengthen the studies. The markers will aim to highlight the articular chs alone, MSCs alone, and co-cultured samples GAG and col II concentrations among other indicators as well.

Yang et al, goes on to explain the importance of considering co-culture pre-treatment to respectively stimulate higher chondrogenesis potential for MSCs. Nazepour & Van Wie's review highlights that certain supplementations such as TGF-B1 can synergistically enhance ACAN and Coll II, however also induce hypertrophy.

Further research is required to highlight the best supplementation, ensure reduced invasive procedures and ensure reduced patient site morbidity to strengthen results as there is still evident varying conclusions in a significant amount of research papers. Nazempour & Van Wie, highlighted that this may be attributed to donor variability, handling techniques, age or even perhaps unsuspected variations in preparation concentrations. More solid confirmatory comparative studies are required before these protocols are to be utilized in practice.

3.5 Scaffolding systems

As highlighted above cartilage is incapable of regenerating itself and current methodology has yet to find a successful treatment plan to allow return to full physiological function (Calabrese et al., 2017). The embedment of chs and MSCs into a cell based three dimensional (3D)scaffold system provides promising results for functional cartilage repair, especially full thickness defects in cartilage tissue(Wang, Blasioli, Kim, Kim, & Kaplan, 2006). A scaffold is a 3D structure proficient in supporting cell establishment, proliferation, and differentiation of cells. Bioactive materials and stimuli are applied to stimulate the cells to differentiate into mature chondrocytes by mimicking the *in vivo* cartilage environment (Calabrese et al., 2017). Articular chs and MSCs lose their original phenotype and receptor abilities when in a monolayer culture, thus a 3D scaffold environment provides a higher cell survival environment (Nazempour & Van Wie, 2016). Several biodegradable and biocompatible material have been experimentally trailed with the main functional aim to provide a 3D scaffold system that mimics the natural extracellular matrix and in vivo environment(Zhang, Zheng, Fan, & Zhang, 2017).

Collagen is a biocompatible and easily obtainable cellular product that provides low immunogenicity and optimal formability. Collagen has been developed into two viable biological scaffold systems; a collagen sponge and a collagen hydrogel. The collagen sponge result in cell adhesion, while the hydrogel protects from cell leakages(L. Zhang et al., 2017). Collagen is limited by its mechanical properties, specifically low tensile and compressive strength and stiffness;

however, crosslinking with natural or synthetic polymers or inorganic materials leads to more promising biomechanics. Collagen remains the most commonly used scaffolding system (L. Zhang et al., 2017).

Wang et al, demonstrated that silk fibroin is now a viable option also for cartilage tissue engineering. Silk is a naturally obtainable degradable protein (isolated from silk worm cocoons of bombyx mori(D. K. Kim, In Kim, Sim, & Khang, 2017) that is biocompatible, has excellent mechanical properties and its processability proves to be a strong candidate for skeletal tissue engineering. Silk is however limited by its oxidation of the fibroin protein at several possible sites, including; side chains, polypeptide backbone or the N-terminal residues (D. K. Kim et al., 2017). Wang et al's studies have demonstrated that MSCs and human chs attach to the aqueous derived silk fibroin scaffold more slowly (up to three hours for attachment) compared to other tissue cultured plastics (TCP) (Wang et al., 2006). The TCP performance was still evidently more superior, as the TCP supported more initial proliferation as well (Wang et al., 2006).

Micro fibrous scaffold systems have been widely utilized due to their natural extracellular matrix resemblance and their ability to encourage cellular activity (attachment and proliferation) due to their resemblance to extracellular matrix. Polycaprolactone (PCL) Is a refined product of the original micro fibrous product, due to limiting mechanical properties such as, low controllability of shapes and pore structure(M. S. Kim & Kim, 2014).

PCL is currently under scientific investigation to identify its candidacy for in vivo tissue engineering implantation application. It is a highly compatible aliphatic (open carbon organic polyester compound) created by polymerization of open looped Ɛ-caprolactone. Is displays excellent biomechanical properties and slow degradation (Su et al., 2018). The porous structure of PCL allows for neo-vasculation and therefore the exchange of nutrients and waste products within the scaffold. The feasibility to utilize this fabricated designed scaffold system for tissue engineering of various hard tissue is highly likely (M. S. Kim & Kim, 2014).

These novel designed scaffold systems have clearly exhibited good bioactivity in vitro, but the applied effects in vivo requires further in vivo clinical animal studies to fully elucidate the clinical relevance.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5288372/

https://www.sciencedirect.com/science/article/pii/S0142961217301023

4.0 Conclusion

Stem cell development is a new and exciting field, especially regarding the treatment of joint disease in the horse. There have been numerous peer reviewed studies published detailing the utilization of mesenchymal stem cells (MSCs) from numerous biological sites for musculoskeletal disease in horses and humans. Favourable results have been

obtained from the co-culture of MSCs with chondrocytes (Chs) for cartilage repair application in humans as well as animal models. Chondrocytes and mesenchymal stem cells produce a synergistic effect on proliferation, gene expression and production of an extracellular matrix like that of native cartilage.

Allogenic MSCs utilization requires provides attractive potential, due to their immediate application at the time of the tissue injury or disease diagnosis. However further studies with controlled MHC responses are required for allogenic MSC therapy before it can be a viable option for treatment going forward. Work in this area is aimed at defining the optimal characteristics and developing a readily available cell source with ideal properties. Allogeneic sources of mature differentiated cells types like Chs are more problematic due to donor-recipient reaction. The procedure to collect autologous articular Chs involves a general anaesthetic and an invasive procedure to obtain articular cartilage, which makes this highly impractical, ethically and financially. Allogenic tissue engineering with Chs requires further research to allow further utilization of these non-immune privileged cells to reduce the number of invasive procedures and general anaesthetics required.

There is a large gap in present knowledge and development of protocols for the obtainment of nasal Chs in horses and their co-culturing with MSCs (both autogenic and allogenic) for tissue engineering application. The use of nasal Chs would reduce the requirement for general anaesthetics. However, there is also a requirement to evaluate if nasal Chs contain equivalent chondrogenic properties as those obtained from articular cartilage for co-culture application for cartilage tissue engineering.

The objective of this literature review was to summarise the current published work on co-culture of MSCs and chondrocytes, with special attention to different Ch sources (i.e. articular vs. nasal). To evaluate the co-culture abilities of allogeneic equine umbilical cord blood derived and autogenic bone-marrow derived MSCs with nasal and articular Chs with further side evaluation of nasal Chs vs articular cartilage performance.

5.0 References

Akers, R. M., & Denbow, D. M. (2013). *Anatomy and physiology of domestic animals. [electronic resource]*: Ames, Iowa : John Wiley & Sons, Inc., 2013

Second edition.

Berglund, A. K., Fortier, L. A., Antczak, D. F., & Schnabel, L. V. (2017). Immunoprivileged no more: measuring the immunogenicity of allogeneic adult mesenchymal stem cells. *Stem Cell Research & Therapy, 8*(1), 288. doi:10.1186/s13287-017-0742-8

Bieback, K., Kern, S., Kocaomer, A., Ferlik, K., & Bugert, P. (2008). Comparing mesenchymal stromal cells from different human tissues: bone marrow, adipose tissue and umbilical cord blood. *Biomed Mater Eng, 18*(1 Suppl), S71-76.

Calabrese, G., Forte, S., Gulino, R., Cefali, F., Figallo, E., Salvatorelli, L., . . . Giuffrida, R. (2017). Combination of Collagen-Based Scaffold and Bioactive Factors Induces Adipose-Derived Mesenchymal Stem Cells Chondrogenic Differentiation In vitro. *Front Physiol, 8*, 50. doi:10.3389/fphys.2017.00050

Chu, C. R., Szczodry, M., & Bruno, S. (2010). Animal models for cartilage regeneration and repair. *Tissue Engineering - Part B: Reviews, 16*(1), 105-115. doi:10.1089/ten.teb.2009.0452

Colbath, A. C., Frisbie, D. D., Dow, S. W., Kisiday, J. D., McIlwraith, C. W., & Goodrich, L. R. (2017). Equine Models for the Investigation of Mesenchymal Stem Cell Therapies in Orthopaedic Disease. *Operative Techniques in Sports Medicine, 25*(1), 41-49. doi:https://doi.org/10.1053/j.otsm.2016.12.007

Foldager, C. B. (2013). Advances in autologous chondrocyte implantation and related techniques for cartilage repair. *Dan Med J, 60*(4), B4600.

Fossum, T. W., Duprey, L. P., & Huff, T. G. (2013). *Small animal surgery*: St. Louis, Missouri : Elsevier Mosby, c2013

4th ed.

Kafienah, W., Jakob, M., Demarteau, O., Frazer, A., Barker, M. D., Martin, I., & Hollander, A. P. (2002). Three-dimensional tissue engineering of hyaline cartilage: comparison of adult nasal and articular chondrocytes. *Tissue Eng, 8*(5), 817-826. doi:10.1089/10763270260424178

Kern, S., Eichler, H., Stoeve, J., Klüter, H., & Bieback, K. (2006). Comparative analysis of mesenchymal stem cells from bone marrow, umbilical cord blood, or adipose tissue. *Stem Cells (Dayton, Ohio), 24*(5), 1294-1301.

Kim, D. K., In Kim, J., Sim, B. R., & Khang, G. (2017). Bioengineered porous composite curcumin/silk scaffolds for cartilage regeneration. *Materials Science and Engineering: C, 78*, 571-578. doi:https://doi.org/10.1016/j.msec.2017.02.067

Kim, M. S., & Kim, G. (2014). Three-dimensional electrospun polycaprolactone (PCL)/alginate hybrid composite scaffolds. *Carbohydrate Polymers, 114*, 213-221. doi:https://doi.org/10.1016/j.carbpol.2014.08.008

McIlwraith, C. W., Fortier, L. A., Frisbie, D. D., & Nixon, A. J. (2011). Equine Models of Articular Cartilage Repair. *Cartilage, 2*(4), 317-326. doi:10.1177/1947603511406531

Nazempour, A., & Van Wie, B. J. (2016). Chondrocytes, Mesenchymal Stem Cells, and Their Combination in Articular Cartilage Regenerative Medicine. *Annals Of Biomedical Engineering, 44*(5), 1325-1354. doi:10.1007/s10439-016-1575-9

Pelttari, K., Pippenger, B., Mumme, M., Feliciano, S., Scotti, C., Mainil-Varlet, P., . . . Martin, I. (2014). Adult human neural crest-derived cells for articular cartilage repair. *Sci Transl Med, 6*(251), 251ra119. doi:10.1126/scitranslmed.3009688

Sjaastad, O. V., Hove, K., & Sand, O. (2016). *Physiology of domestic animals*: [Oslo, Norway] : Scandinavian Veterinary Press, [2016]

Third edition.

Su, Y., Denbeigh, J. M., Camilleri, E. T., Riester, S. M., Parry, J. A., Wagner, E. R., . . . Kakar, S. (2018). Extracellular matrix protein production in human adipose-derived mesenchymal stem cells on three-dimensional polycaprolactone (PCL) scaffolds responds to GDF5 or FGF2. *Gene Reports, 10*, 149-156. doi:https://doi.org/10.1016/j.genrep.2017.12.004

Vo, N., Niedernhofer, L. J., Nasto, L. A., Jacobs, L., Robbins, P. D., Kang, J., & Evans, C. H. (2013). An overview of underlying causes and animal models for the study of age-related degenerative disorders of the spine and synovial joints. *Journal of Orthopaedic Research, 31*(6), 831-837. doi:10.1002/jor.22204

Wang, Y., Blasioli, D. J., Kim, H.-J., Kim, H. S., & Kaplan, D. L. (2006). Cartilage tissue engineering with silk scaffolds and human articular chondrocytes. *Biomaterials, 27*(25), 4434-4442. doi:https://doi.org/10.1016/j.biomaterials.2006.03.050

Zhang, H., Tao, Y., Liu, H., Ren, S., Zhang, B., & Chen, H. (2017). Immunomodulatory function of whole human umbilical cord derived mesenchymal stem cells. *Molecular Immunology, 87*, 293-299. doi:https://doi.org/10.1016/j.molimm.2017.03.003

Zhang, L., Zheng, L., Fan, H. S., & Zhang, X. D. (2017). A scaffold-filter model for studying the chondrogenic differentiation of stem cells in vitro. *Materials Science and Engineering: C, 70*, 962-968. doi:https://doi.org/10.1016/j.msec.2016.04.015

Zuo, Q., Cui, W., Liu, F., Wang, Q., Chen, Z., & Fan, W. (2013). Co-cultivated mesenchymal stem cells support chondrocytic differentiation of articular chondrocytes. *International Orthopaedics, 37*(4), 747-752. doi:10.1007/s00264-013-1782-z

4.0 Appendix

Appendix A

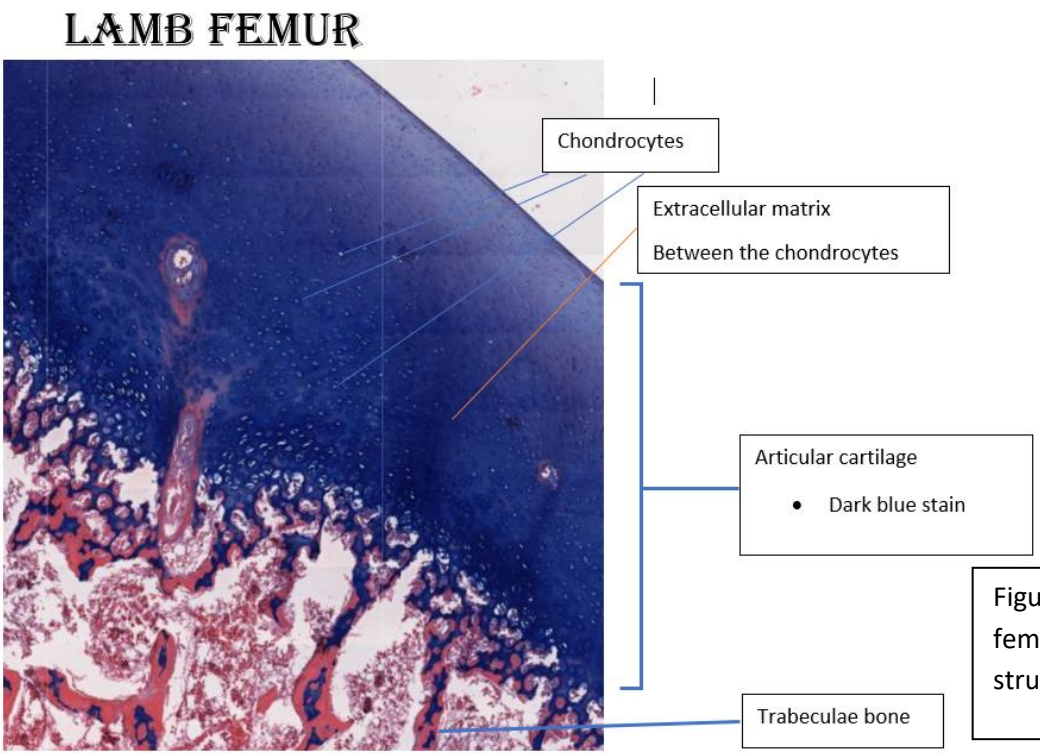

Figure 1a: Histological photo of Lamb femur-highlighting the histological structure of articular cartilage

Appendix B

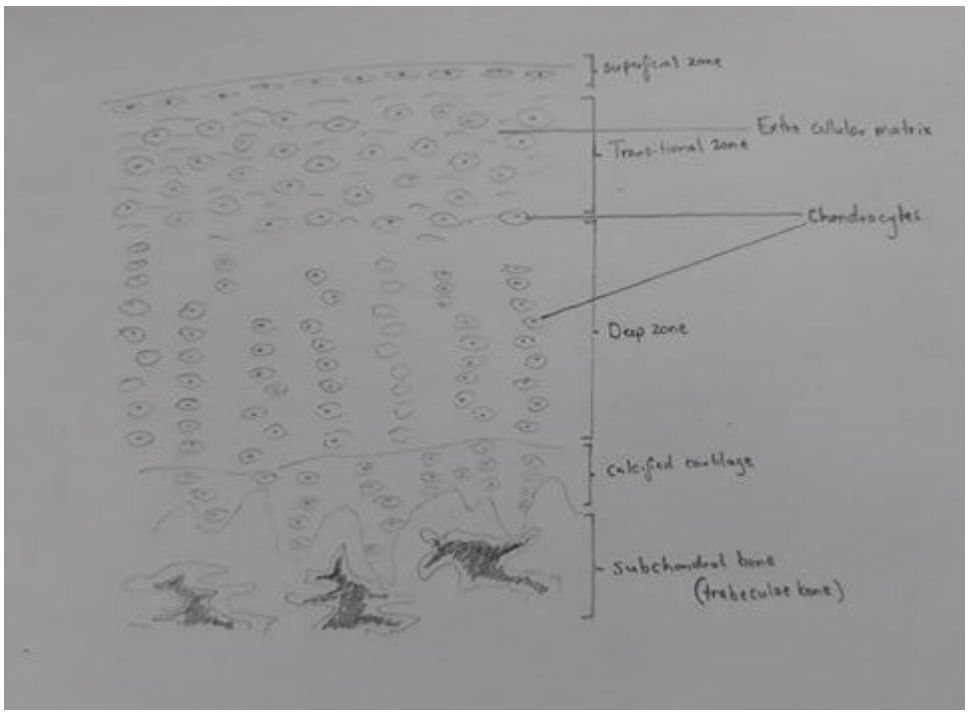

Figure 1b: Histological drawing of Lamb femur-highlighting the histological structure of articular cartilage. Illustrated by Samantha Looney, 2017