CHIEF SCIENTIST OFFICE

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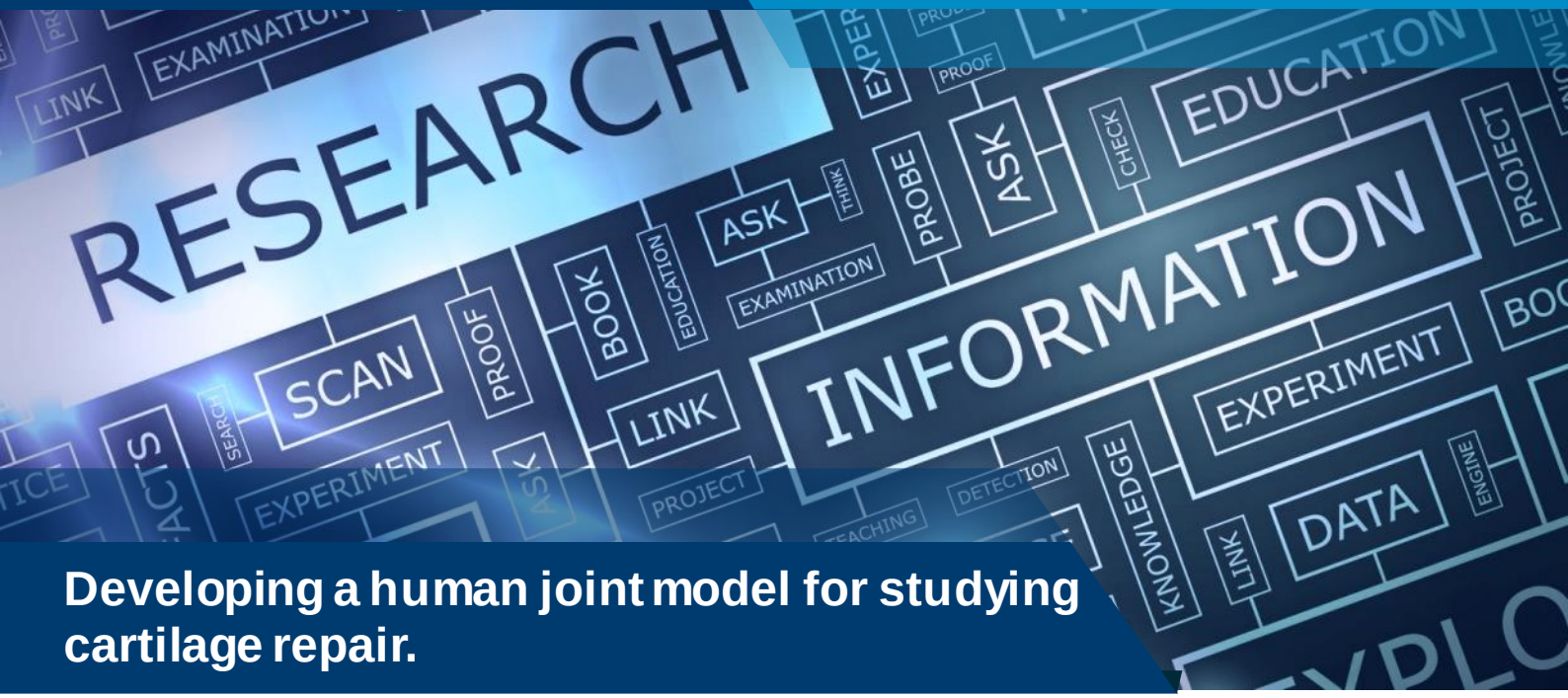
RESEARCH PROJECT BRIEFING



Developing a human joint model for studying cartilage repair.

AIMS

In the U.K. each year, 4.7m patients aged 45-64yrs seek treatment for osteoarthritis of the hip, knee and ankle. Non-surgical treatment includes painkillers, physiotherapy and steroids. Surgical treatment is usually joint replacement or permanent joint fixing. As osteoarthritis is so common, treating the disease represents a huge socio-economic healthcare burden due to the disability and cost of treatment. Job-related osteoarthritis is more costly than asthma/pulmonary diseases and renal/neurologic diseases combined.

In osteoarthritis, the cartilage covering the joints becomes damaged and worn away revealing the bone underneath which, when the joint is used, is very painful. Cartilage is incapable of repair and surgeons usually replace the joint which is expensive. It would be much better if we could inject material onto the femoral head to repair the damaged cartilage and restore joint function. However, first of all we need a good human joint model on which we can test these materials.

The best joint is the femoral head which is a ball-and-socket type with the 'ball' being called the femoral head and the socket being the cup into which the femoral head articulates. Often during a fall, the bone in the hip joint breaks, meaning that surgery has to be performed to fit a replacement (metal) hip joint. This is called a femoral neck fracture, and the broken-off ball, or femoral head, is usually thrown away as it cannot be fitted back onto the broken bone. However these femoral heads are often in good condition and are very useful for research into osteoarthritis. The aim of this project was to develop this human femoral head model so that (1) we could keep the femoral head alive for several weeks, (2) we could make small cavities in the cartilage into which repair material could be inserted and (3) we could see if some special cells could fill the cavities with cartilage repair material.

KEY FINDINGS

- We developed methods enabling human femoral cartilage to be kept alive for up to 10 weeks.
- We prepared small cavities or 'wells' in the cartilage that could be filled with 'repair' material.
- We optimised procedures so that cartilage repair could be studied over several weeks.
- We performed pilot experiments with special cells (mesenchymal stromal cells) to see if they could fill the cavities with cartilage.
- We established an excellent method termed a 'human femoral head model' which can now be used for testing techniques for repairing human articular cartilage damaged as a result of osteoarthritis.

WHAT DID THE STUDY INVOLVE?

This study involved obtaining fresh human femoral heads at surgery by clinical colleagues from patients with femoral neck fracture. The patients were informed by our research nurse about the nature of the project and the fact that the femoral heads removed during surgery were otherwise going to be thrown away but could be very useful for our research. We only used femoral heads obtained following patients' consent. The Ethical permission required for this project was obtained from the NHS Ethics Board. Patients were thanked for the donation.

During surgery, the femoral heads were placed in a cold solution, and quickly delivered to our Research laboratories. When fresh femoral heads arrived, first of all we had to assess whether the viability of the cartilage cells (chondrocytes) was high enough to justify these time-consuming long-term experiments. Small cartilage samples from the femoral head were taken, and the cartilage cells (chondrocytes) labelled with special dyes and studied for living cells using an advanced microscope. The femoral heads were occasionally damaged as a result of the patient suffering from femoral neck fracture, and there were also problems with infection which killed the chondrocytes meaning that frequently the femoral head was not suitable for further study. Nevertheless, healthy femoral heads were obtained with an initial chondrocyte viability of better than 95%. By removing cartilage explants on a weekly basis, we monitored the viability of the chondrocytes under various conditions for up to 10 weeks.

WHAT WERE THE RESULTS AND WHAT DO THEY MEAN?

Having established the conditions under which we could keep the femoral head and its cartilage alive, we now sought to test a range of conditions to maintain the high viability of the cartilage cells over the 10 week period. As this research had not been done before by anyone else, we had to test a range of conditions including different nutrient media with various additives, and how often the solutions were mixed and refreshed. We found that when the femoral heads were cultured in a standard glucose-containing solution with human serum (without mixing during culture), cell viability was high throughout the 10 week period (Fig. 1A). However some conditions tested caused a rapid reduction in chondrocyte viability and were therefore unsuitable for these studies (Fig. 1B).

The next step was to determine the optimal size and shape of the small 'wells' in the cartilage into which the repair material which included special cells could be placed. This was to ensure that we could test as many conditions as possible but not cause too much damage to the femoral heads because of handling and cutting. The idea was to make a hole in the cartilage and then stimulate the cells to make new cartilage thereby filling the hole. To identify the optimal size for the cartilage wells, we tested a number of different methods and found that a small (5mm) biopsy punch was ideal to create a reasonable number of 'wells' suitably spaced out across the femoral head (Fig. 1C).

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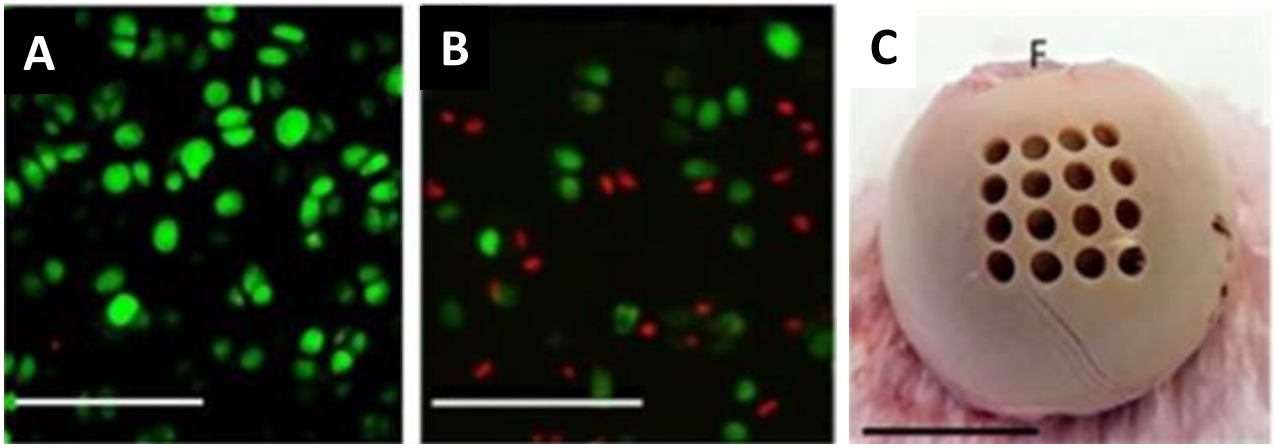

Figure 1. The human *ex vivo* femoral head model and viability of cartilage cells (chondrocytes) following culture. (A) Shows the viability of human chondrocytes after 10 weeks in culture medium +10% human serum, and (B) shows the effects of culture medium + 10% human serum with mixing. (C) Shows the femoral head with cartilage 'wells' prepared using a 5mm biopsy punch. F identifies the fovea from which a ligament emerges and is attached to the acetabulum. Scale bar = 25mm. (Green label = living chondrocytes; red label, dead chondrocytes). Scale bar = 100µm.

Having optimised the conditions for culture and the preparation of the 'cartilage wells', we then prepared special cells (mesenchymal stromal cells. MSCs) and inserted them into the cartilage wells and cultured the femoral heads under optimal conditions. We used these cells because they are relatively 'simple' cells and given the right signals, can be directed to form cartilage cells and hence attempt to repair the hole in the cartilage. At various time points, we carefully cut around the wells and removed them for the detailed study of the cells and the cartilage produced to see if they were generating a repair cartilage to fill the experimental wells. Our preliminary data with MSCs demonstrated the early stages of a 'repair' process with a thin film of cells and tissue spreading across the cartilage well in the cultured femoral heads (Fig. 2). An analysis of the 'repair' tissue using methods we have developed, showed some properties expected of cartilage and preliminary evidence of integration of the repair tissue with the surrounding native cartilage. This is important because it is essential that the repair tissue is retained within the well and would not be dislodged. These results mean that we have established a long-term viable human femoral head model which can then be used to test a wide range of pre-clinical strategies and therapies for the repair of human cartilage.

WHAT WERE THE RESULTS AND WHAT DO THEY MEAN?

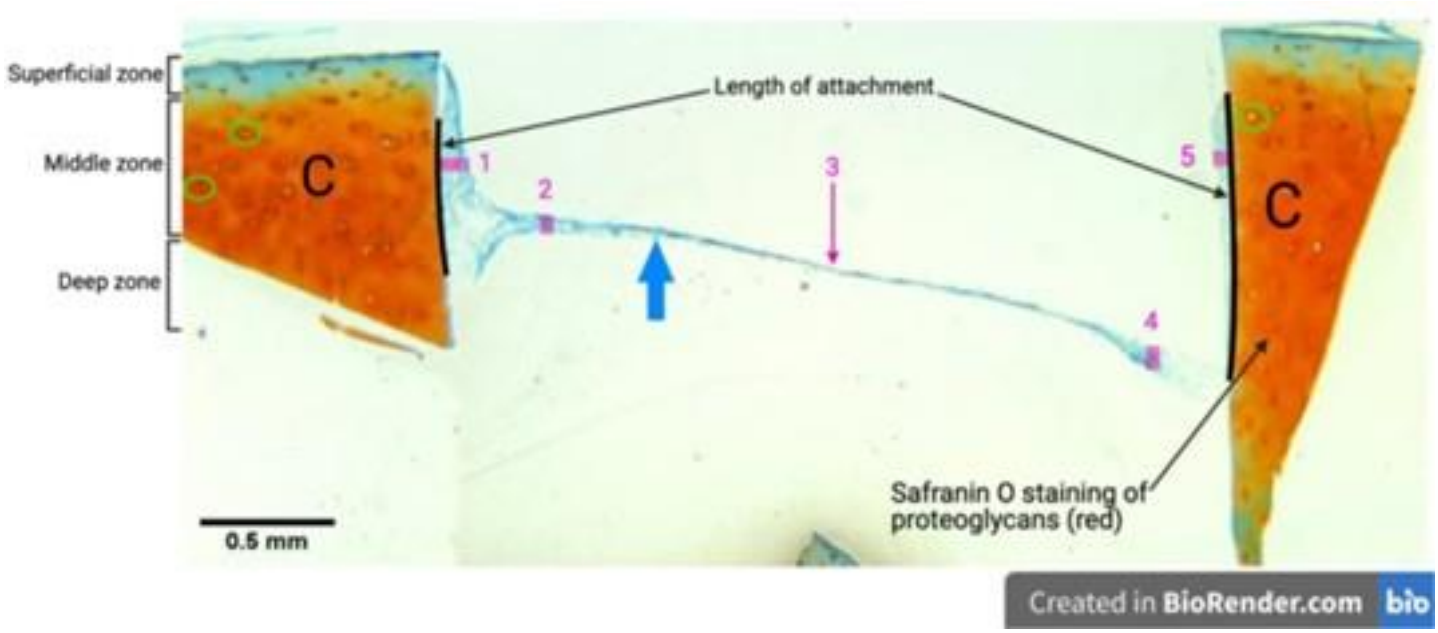

Figure 2. A cross section of a cartilage 'well' in the *ex vivo* human femoral head model showing the presence of a repair 'film' of mesenchymal stromal cells (MSCs). The cartilage at the edges of the well are shown as **C** with the zones (superficial, mid- and deep) on the left hand side. The proteins of the cartilage are stained with safranin O (red/orange) for clarity. The mesenchymal stromal cells (MSCs) have formed a thin film across the cartilage well, and its thickness quantified at the 5 points identified, along with the length of the attachments (thick black lines) adjacent to the native cartilage. Scale bar = 0.5mm.

We think that these results are important in the field of cartilage research as they describe a viable and straightforward model and experimental method for investigating human cartilage repair. The technique could be further adapted to study treatment options for injured cartilage and potentially to culture whole osteoarthritic human femoral heads. It should be noted that we believe our model is unique as the vast majority of research in this field uses an animal model for assessing cartilage repair. There is concern amongst many scientists that animal models may not be appropriate for understanding osteoarthritis and cartilage repair in human joints. A full description of the model has been published (Styczynska-Soczka et al., 2020; 2021).

WHAT IMPACT COULD THE FINDINGS HAVE?

This study has identified a human cartilage model that will have significant benefit as a test bed for determining a range of methods for cartilage repair. The successful methods for cartilage repair could then be taken for clinical testing in patients with osteoarthritis.

HOW WILL THE OUTCOMES BE DISSEMINATED?

The results of this study have already been communicated as oral and posters at live and virtual meetings of the Bone Research Society (BRS) and the British Orthopaedic Research Society (BORS). We have also published the work in two research papers in high impact peer-reviewed journals *Cartilage* and the *Journal of Cellular Physiology*. We will also present work at the forthcoming meeting of the International Combined Orthopaedic Research Society (September 2022, Edinburgh).

CONCLUSION

This project succeeded in its aims and has developed an *ex vivo* model of the human femoral head for studying cartilage repair. The details of this model have been published and are available for use by other groups. The next stage will be to obtain funding to identify strategies for signalling the mesenchymal stromal cells to fill and repair the cartilage wells by producing a tough cartilage which integrates with the surrounding healthy cartilage. Securing future funding is essential to realise the benefits of this approach and therefore applications are on-going. This project represented an important and essential first step in the long journey to identify methods for human cartilage repair.

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Additional Information:

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