

## Author's Reply to the Review Report 2

We are grateful to the reviewer for their positive and constructive comments that have led to an improved manuscript. Here, we address only their concerns/suggestions and have tracked changes throughout the revised manuscript.

### Introduction:

**Point 1:** Text surrounding references [6,7] – it is suggested to cite some high quality, recent (<3 years) review papers on osteochondral scaffolds/devices to better illustrate this point.

**Response 1:** Two recent papers have been added to the text (lines 46-48): Urbanek, O., D. Kołbuk, and M. Wróbel, 2019 focussing primarily on the challenges of articular cartilage regeneration and devices and regeneration and Ansari, S., S. Khorshidi, and A. Karkhaneh, 2019 focusing more widely on osteochondral devices and regeneration.

**Point 2:** References [11,12] – these two references are both very old and do not reflect the current state of the literature, please replace with more recent references.

**Response 2:** These references to micromotion have been replaced on lines 53-54 with a more recent study.

**Point 3:** More details on the principles and processes involved need to be given on how micro-CT imaging and DVC can be used to derive non-destructive mechanical evaluation of tissues.

**Response 3:** We agree that an introduction to the principals of micro-CT and DVC is necessary. Images captured with 3D imaging techniques such as micro-CT are combined with DVC to track features within a sample between a reference state and during mechanical loading. Features are correlated between the two or more loading states resulting in displacement vectors from which strains can be derived, non-destructively in 3D. DVC was initially developed for use with micro-CT scanning of trabecular bone 20 years ago, utilising the trabecular microarchitecture as features to create a unique pattern. The development of the technique enabled non-destructive mechanical evaluation of the tissue and ultimately provided full-field 3D strain maps for the first time.

Detail has been added to introduce the principles and process of micro-CT and DVC (lines 66-72) including the addition of a reference to the initial study which developed the technique (Bay et al.).

**Point 4:** The introduction should briefly explain the study design – why was the sheep model (and only 1 animal) chosen? What is the defect representative of and what is the justification of the implant material/morphology used?

**Response 4:** The ovine model was chosen because it is regularly used for the development of articular cartilage devices. Thus it was the best model for us to demonstrate our method of strain measurement. Reference to previous use of the sheep model has been added to the introduction (line 83).

Only one animal was included for the presented study because the technique was previously unproven and thus it was unfeasible for a large animal study to include a larger sample size at that time. It should be noted that the aim of the study was to prove the micro-CT/DVC method works in this application, rather than to demonstrate our scaffold material was suitable to regenerate cartilage. Text has been added to clarify the aim, and main findings, of the study (lines 316-319) and includes how our work has progressed the field (lines 339-340). DVC studies of a similar nature, but in the much more heavily studied research area of bone, have recently been published with similar sample sizes. Recently published examples include: Peña Fernández et al, ACS Biomater. Sci. Eng, 2019 (n = 1), Madi et al, Nature Biomed. Eng., 2019 (groups of n =1 and n = 2), Peña Fernández et al, Materials, 2020 (n = 2). Our study is the first to apply the method to an osteochondral defect. The defect is representative of an osteochondral repair of focal cartilage damage.

The implant material choice is based on the ability to create a scaffold with similar mechanical properties to cartilage, degradable, withstand cyclic loading in the body and early lab data that showed when human mesenchymal stem cells were sent down a chondrogenic lineage and produced articular-like cartilage *in vitro*. Text has been added to the introduction describing rationale for choosing this implant include references to previous work (lines 81-89).

#### Materials and methods:

**Point 5:** The sentence before the start of the first sub-section is a mistake and should be removed.

**Response 5:** This text has been removed.

**Point 6:** There needs to be a more detailed description of the custom-built mechanical testing equipment in the methods text on page 4, corresponding to Figure 1d. The components of the equipment shown in Figure 1d have not been explained in the text.

**Response 6:** A substantially more detailed description for the components of the *in-situ* testing rig corresponding to Figure1d have been added to page 4 (lines 162-171).

#### Results and discussion:

**Point 7:** The results and discussion are relatively meaningless as it is obvious that the implant did not integrate with the host tissue and sank below the chondral surface. The loading was not even conveyed to the implant and only one sample was tested. All of the ensuing analyses on mechanical parameters are therefore based on an invalid model. It is not clear if the findings of this study contribute any useful information to the current literature and innovation is very low. There needs to be at least several samples with proper placement for a valid analysis.

**Response 7:** The intent of our study was to demonstrate the DVC/micro-CT method can measure strains in an osteochondral defect-implant system and this is the first time this has been done. In this respect, the study has made three valuable contributions to the literature: establishing a sample preparation and micro-CT scanning protocol for this tissue-biomaterial system, micromotion analysis and error quantification.

Micro-CT scanning: we provide an optimised protocol, using only laboratory micro-CT, which would be of use to future researchers. A comparison to our initial non-optimised scan is shown in Figure 7. Further reference to this has been added (lines 318/319).

Micromotion: Displacement and thus relative micromotion of the implant were successfully detected (Figure 5). The non-destructive 3D measurement of micromotion itself is of great importance with published studies such as one using DVC on an ex-vivo porcine bone-implant system (Sukjamsri et al.) which we have now referenced (lines 338-340). No such studies previously existed for implants across the osteochondral interface.

Error analysis: A large part of DVC publications focus on error analysis (Tozzi et al., Materials 2020) and published DVC studies often only include an error analysis with no loading data such as: Pena Fernandez et al., Microscopy, 2018, Wang et al., Experimental Mechanics, 2018 and Buljac et al., J Strain Analysis, 2018. Therefore in this context the study provides a valuable contribution for the DVC community. Further comparison to literature is now included in the discussion (lines 354-372).

Hopefully, reviewer will see the contributions to the literature are related to the DVC method, rather than advocating the implant material/design: if that were the case we would not have submitted this work for exactly the reasons stated by the reviewer.

**Point 8:** There is no control for this study – why not conduct the same experiment using the contralateral leg as a comparison? Also, since the level of healing was not assessed, what is the rationale for implanting the sample for a period of 6 weeks? How might this have impacted the results?

**Response 8:** The aim of the study was to demonstrate the DVC/micro CT method can measure strains in an osteochondral defect, rather than to demonstrate the implant regenerates cartilage, but we accept a contralateral control could provide useful data and have included this as a limitation (lines 387-390).

The period of 6 weeks is a balance of the healing time that we have experienced with prior ovine models against the cost of a longer study. As the goal was to demonstrate the strain measurement methodology, the time period would not have impacted the results as long as some load had been applied to the joint (which it had). Reference to another six week ovine model study has been added (line 83).