Bone & Joint Research

Supplementary Material

10.1302/2046-3758.1010.BJR-2020-0504.R1

Scaffold fabrication

Briefly, 1% collagen solution was prepared by adding 1 g of lypholized acid-soluble collagen type I (Lando Biomaterials, China) to 100 ml of dilute acetic acid (pH 3.2) and homogenized on ice. The suspension was then crosslinked using 50:17:50 mM EDC:NHS:MES (*N*-Ethyl-*N'*-(3-dimethylaminopropyl) carbodiimide: *N*-hydroxy-succinimide: 2-(*N*-morpholino) ethanesulfonic acid, Sigma-Aldrich, USA) as described previously,[1] and homogenized again after rinsing in 0.1 M Na_2HPO_4 and distilled water. To produce collagen-hydroxyapatite (HAp) (40% and 70%) suspensions, 0.7g and 2.3g of HAp (Sigma-Aldrich) were added slowly during homogenization of the crosslinked collagen suspension. Air bubbles were removed through vacuum degassing. The suspensions were placed layer by layer into a custom-made resin mould (8 mm diameter and 10 mm deep), frozen at -20°C for at least 24 hours and freeze-dried (Christ Alpha 1-2LD, UK) for at least another 24 hours. The scaffolds were then sealed in a plastic bag and sterilized by gamma irradiation (Synergy Health, UK).

References

1. **Tilley JMR, Chaudhury S, Hakimi O, Carr AJ, Czernuszka JT.** Tenocyte proliferation on collagen scaffolds protects against degradation and improves scaffold properties. *Journal of Materials Science-Materials in Medicine*. 2012;23(3):823-833.

2. **John T.** ISIS Physiological Reference Intervals for Captive Wildlife: 913 Species Partitioned by Age and Gender for Large Sample Sizes. Apple Valley, MN: International Species Information System; 2013.

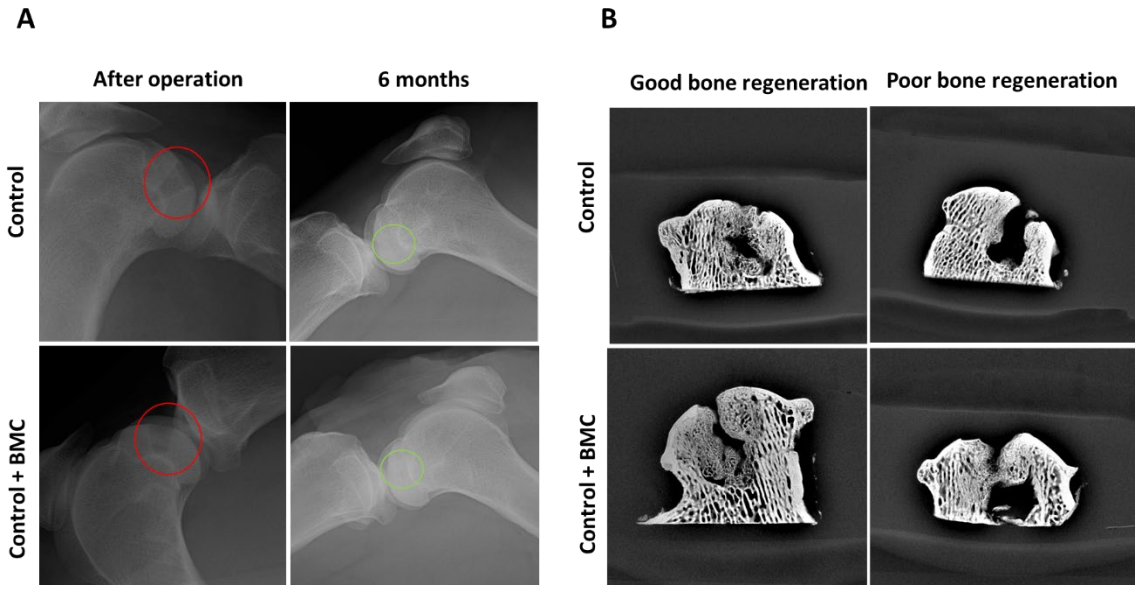

Fig a. a) Lateral radiograph of the joint immediately after the operation, and after six months at euthanasia. b) Lateral representative radiographs of the bone sections with both good and poor bone regeneration. BMC, bone marrow concentrate.

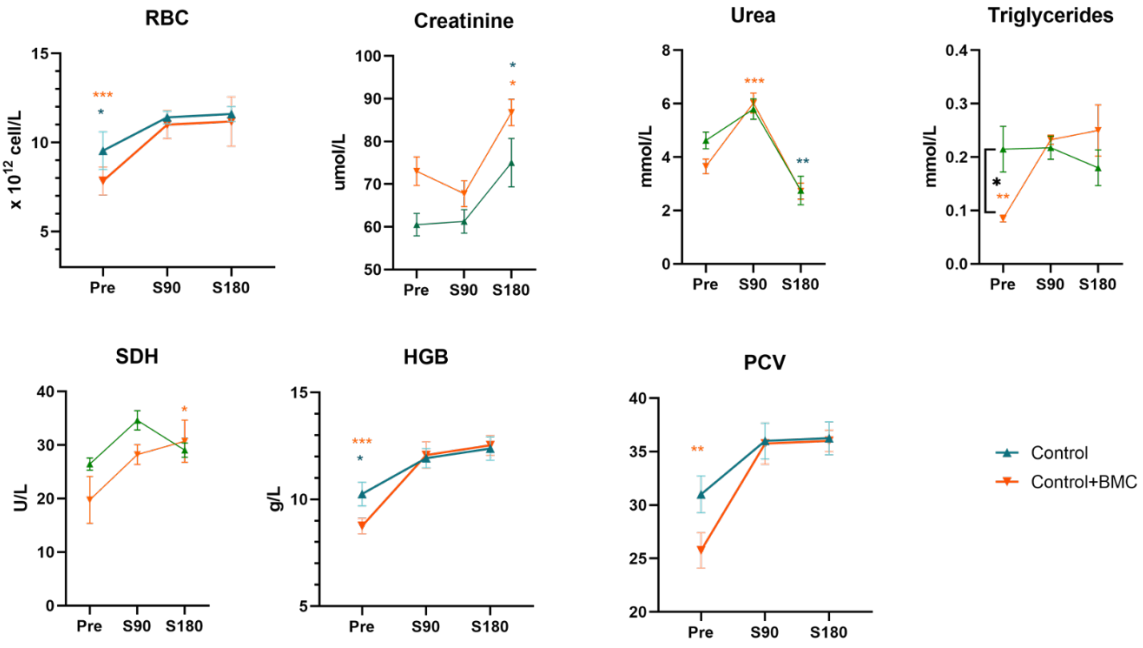

Fig b. Red blood cells (RBCs) increased significantly in both groups over time compared to preoperation; in control group (pre vs S90, p = 0.013 and pre vs S180, p = 0.007) and in control + bone marrow concentrate (BMC) group (pre vs S90, p = 0.0001 and pre vs 180, p < 0.0001). Haemoglobin (HGB) also increased significantly over time, in control group (pre vs S180, p = 0.02) and in control + BMC group (pre vs S90, p =

0.0006 and pre vs S180, p = 0.0002). The same trend was followed for haematocrit (HCT), but packed cell volume (PCV) only increased significantly in control + BMC group (pre vs S90, p = 0.001 and pre vs 180, p = 0.0009). The same was applied to sorbitol dehydrogenase (SDH) levels, which increased significantly only in BMC group (pre vs S180, p = 0.03) and triglycerides levels (pre vs S90, p = 0.009 and pre vs 180, p = 0.004), however, there was a significant difference between the groups before the operation in the latter. There was an increased level of creatinine at S180 compared to previous timepoints in both groups, control (pre vs S180, p = 0.03 and S90 vs S180, p = 0.04) and control + BMC (pre vs S180, p = 0.04 and S90 vs S180, p = 0.004). There was a spike in urea levels at day 90, this was significant in BMC group (pre vs S90, p = 0.0008 and S90 vs S180, p < 0.0001), followed by a sharp decrease at day 180, which was significant in the control group (pre vs S180, p = 0.006 and S90 vs S180, p < 0.0001). In general, there was no significant difference between the groups after 90 and 180 days, and none of the measured levels were outside of the normal range according to John.[2] Error bars: standard error of means.

The ARRIVE Guidelines Checklist

Animal Research: Reporting In Vivo Experiments

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	ITEM	RECOMMENDATION	Section/ Paragraph
Title	1	Provide as accurate and concise a description of the content of the article as possible.	title
Abstract	2	Provide an accurate summary of the background, research objectives, including details of the species or strain of animal used, key methods, principal findings and conclusions of the study.	abstract
INTRODUCTION			
Background	3	a. Include sufficient scientific background (including relevant references to previous work) to understand the motivation and context for the study, and explain the experimental approach and rationale. b. Explain how and why the animal species and model being used can address the scientific objectives and, where appropriate, the study's relevance to human biology.	Section 1
Objectives	4	Clearly describe the primary and any secondary objectives of the study, or specific hypotheses being tested.	Section 1, page 2
METHODS			
Ethical statement	5	Indicate the nature of the ethical review permissions, relevant licences (e.g. Animal [Scientific Procedures] Act 1986), and national or institutional guidelines for the care and use of animals, that cover the research.	Section 2.3
Study design	6	For each experiment, give brief details of the study design including: a. The number of experimental and control groups. b. Any steps taken to minimise the effects of subjective bias when allocating animals to treatment (e.g. randomisation procedure) and when assessing results (e.g. if done, describe who was blinded and when). c. The experimental unit (e.g. a single animal, group or cage of animals). A time-line diagram or flow chart can be useful to illustrate how complex study designs were carried out.	a. Section 2.3 b. Section 2.11 c. Section 2.11
Experimental procedures	7	For each experiment and each experimental group, including controls, provide precise details of all procedures carried out. For example: a. How (e.g. drug formulation and dose, site and route of administration, anaesthesia and analgesia used [including monitoring], surgical procedure, method of euthanasia). Provide details of any specialist equipment used, including supplier(s). b. When (e.g. time of day). c. Where (e.g. home cage, laboratory, water maze). d. Why (e.g. rationale for choice of specific anaesthetic, route of administration, drug dose used).	Section 2.3
Experimental animals	8	a. Provide details of the animals used, including species, strain, sex, developmental stage (e.g. mean or median age plus age range) and weight (e.g. mean or median weight plus weight range). b. Provide further relevant information such as the source of animals, international strain nomenclature, genetic modification status (e.g. knock-out or transgenic), genotype, health/immune status, drug or test naïve, previous procedures, etc.	Section 2.3

Housing and husbandry	9	Provide details of: a. Housing (type of facility e.g. specific pathogen free [SPF]; type of cage or housing; bedding material; number of cage companions; tank shape and material etc. for fish). b. Husbandry conditions (e.g. breeding programme, light/dark cycle, temperature, quality of water etc for fish, type of food, access to food and water, environmental enrichment). c. Welfare-related assessments and interventions that were carried out prior to, during, or after the experiment.	Section 2.3
Sample size	10	a. Specify the total number of animals used in each experiment, and the number of animals in each experimental group. b. Explain how the number of animals was arrived at. Provide details of any sample size calculation used. c. Indicate the number of independent replications of each experiment, if relevant.	a. Section 2.3
Allocating animals to experimental groups	11	a. Give full details of how animals were allocated to experimental groups, including randomisation or matching if done. b. Describe the order in which the animals in the different experimental groups were treated and assessed.	Section 2.11
Experimental outcomes	12	Clearly define the primary and secondary experimental outcomes assessed (e.g. cell death, molecular markers, behavioural changes).	Sections 2.4 to 2.10
Statistical methods	13	a. Provide details of the statistical methods used for each analysis. b. Specify the unit of analysis for each dataset (e.g. single animal, group of animals, single neuron). c. Describe any methods used to assess whether the data met the assumptions of the statistical approach.	Section 2.11
RESULTS			
Baseline data	14	For each experimental group, report relevant characteristics and health status of animals (e.g. weight, microbiological status, and drug or test naïve) prior to treatment or testing. (This information can often be tabulated).	Section 2.3
Numbers analysed	15	a. Report the number of animals in each group included in each analysis. Report absolute numbers (e.g. 10/20, not 50%[2]). b. If any animals or data were not included in the analysis, explain why.	Pages 9-11, the numbers are included
Outcomes and estimation	16	Report the results for each analysis carried out, with a measure of precision (e.g. standard error or confidence interval).	Sections 3.1-3.6
Adverse events	17	a. Give details of all important adverse events in each experimental group. b. Describe any modifications to the experimental protocols made to reduce adverse events.	N/A
DISCUSSION			
Interpretation/ scientific implications	18	a. Interpret the results, taking into account the study objectives and hypotheses, current theory and other relevant studies in the literature. b. Comment on the study limitations including any potential sources of bias, any limitations of the animal model, and the imprecision associated with the results[2]. c. Describe any implications of your experimental methods or findings for the replacement, refinement or reduction (the 3Rs) of the use of animals in research.	Section 4
Generalisability/ translation	19	Comment on whether, and how, the findings of this study are likely to translate to other species or systems, including any relevance to human biology.	
Funding	20	List all funding sources (including grant number) and the role of the funder(s) in the study.	Section 7

References:
1. Kilkenny C, Browne WJ, Cuthill IC, Emerson M, Altman DG (2010) Improving Bioscience Research Reporting: The ARRIVE Guidelines for Reporting Animal Research. *PLoS Biol* 8(6): e1000412. doi:10.1371/journal.pbio.1000412
2. Schulz KF, Altman DG, Moher D, the CONSORT Group (2010) CONSORT 2010 Statement: updated guidelines for reporting parallel group randomised trials. *BMJ* 340:c332.