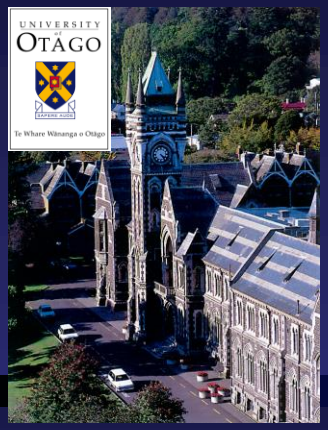
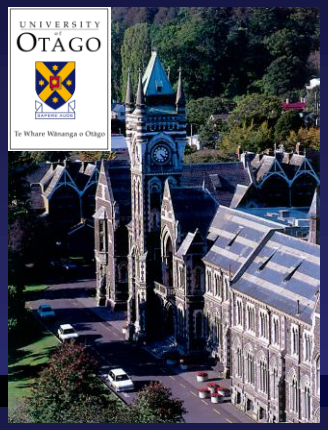


Novel Use of Biomaterials in a Clinical Setting: One Surgeon's Journey from the Clinic to the Laboratory and Back Again

George J Dias

Department of Anatomy, School of Medical Sciences, University of Otago, New Zealand.



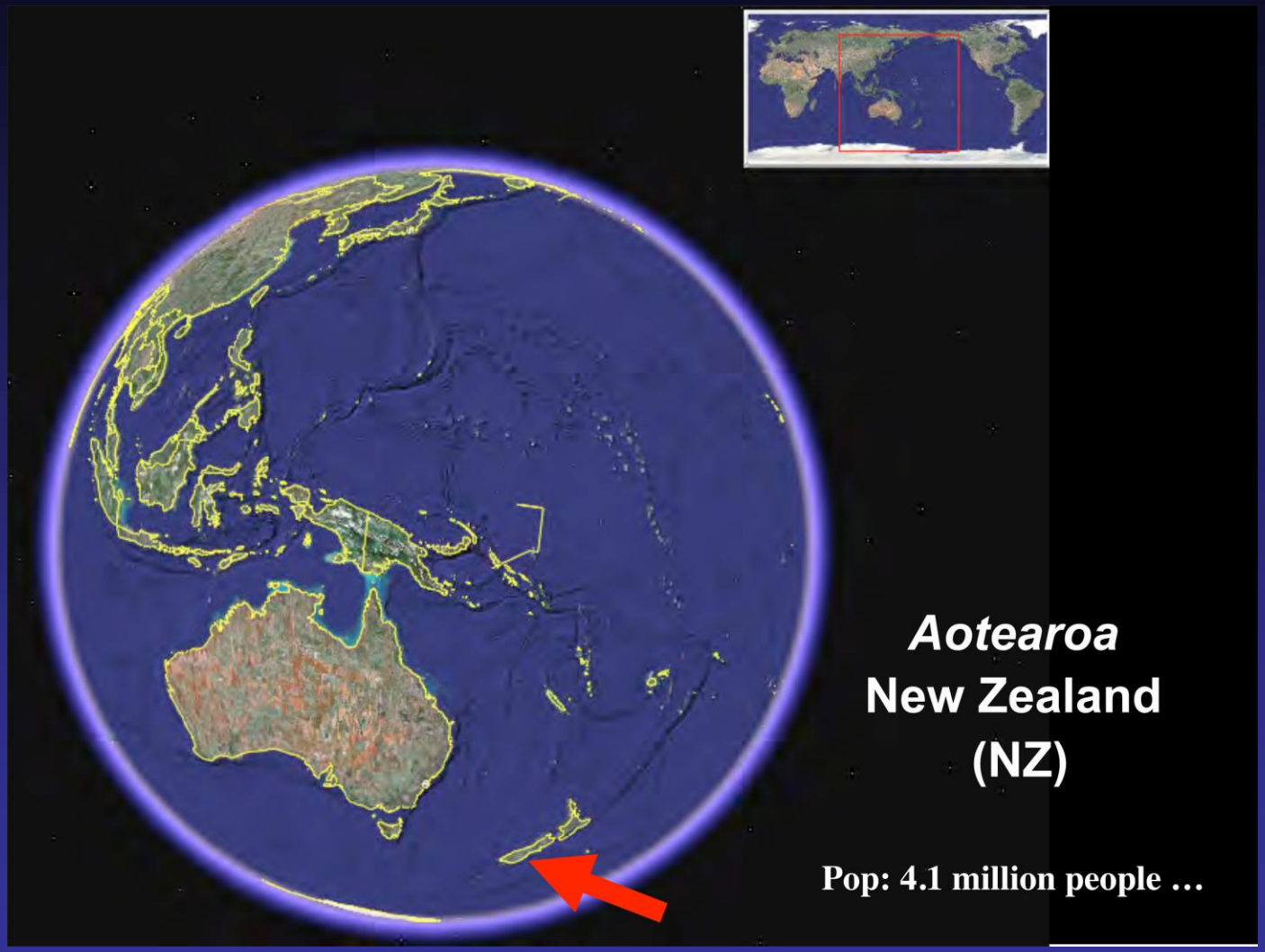
Resorbable keratin-based biopolymer as a bone substitute material

George J Dias

Department of Anatomy, School of Medical Sciences, University of Otago, New Zealand.

AgResearch
Christchurch, New Zealand

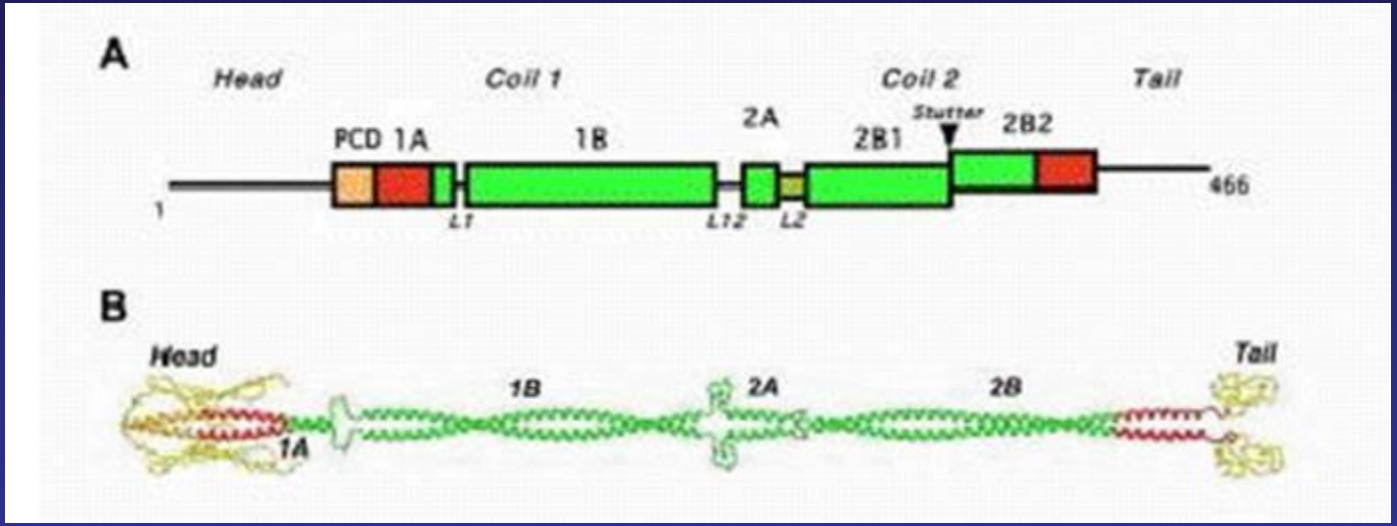
Department of Anatomy, School of Medical Sciences
University of Otago



- … and 38.5 million sheep

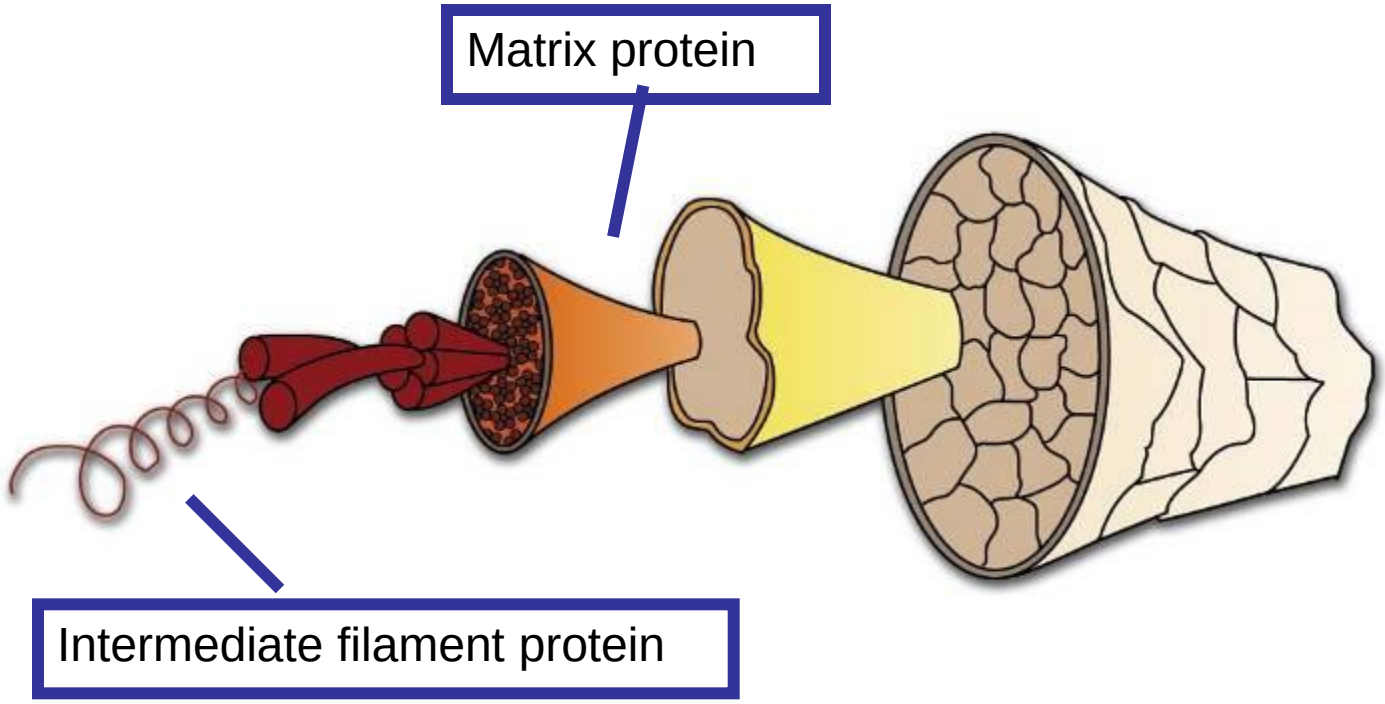
- 2007 Agricultural Production Census, Statistics New Zealand
- NZ is the 3rd largest (17%) global wool producer (after Australia and China), and 2nd largest exporter (after Australia)

- Intact keratin proteins



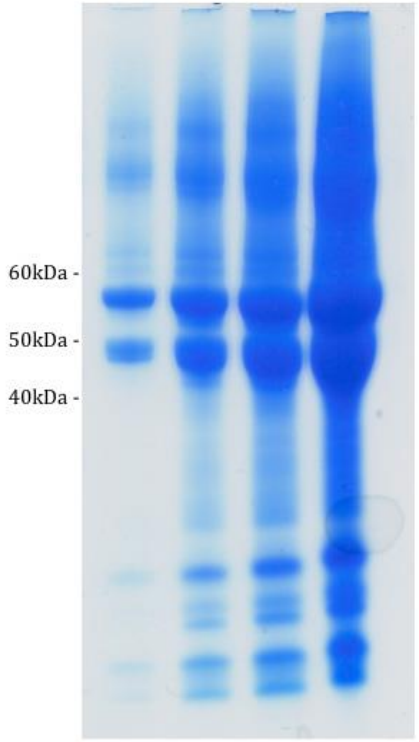
From: Strelkov SV, et. al. 2000

Keratin protein fractions

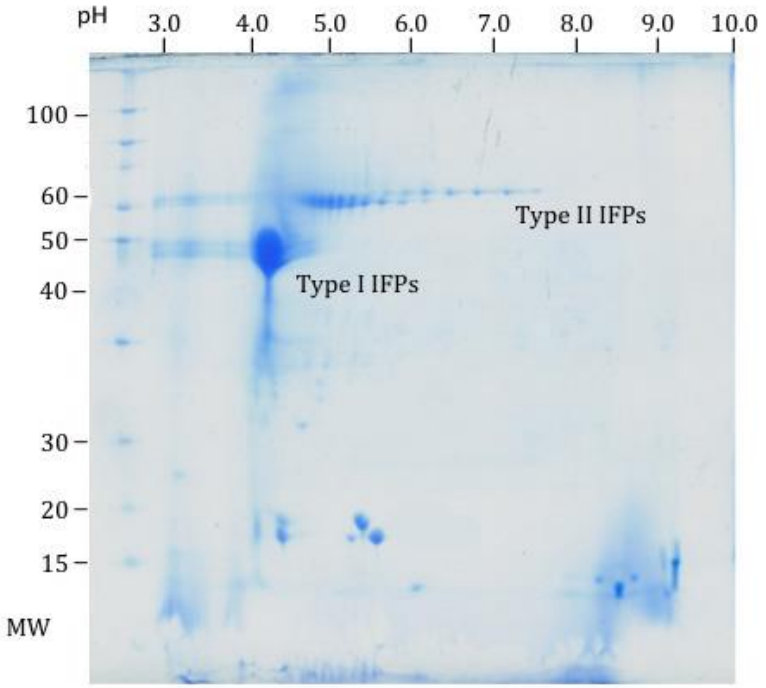


Reversible protection of cystine crosslinks

Electrophoresis

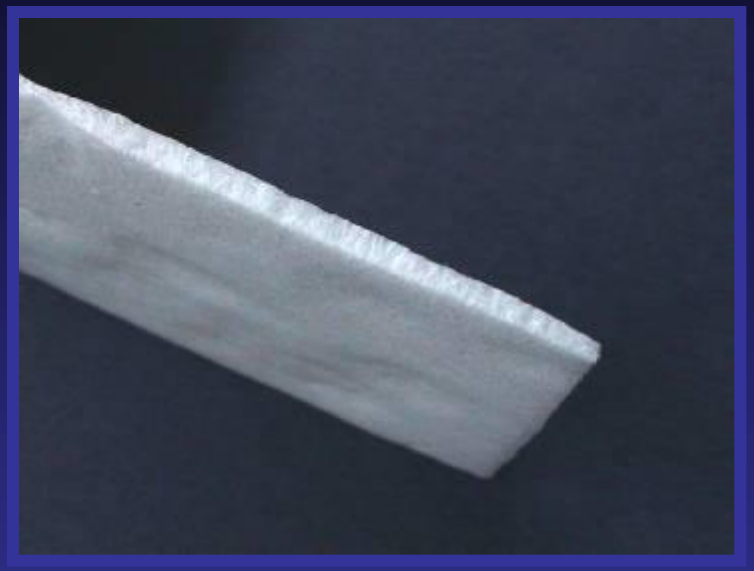


SDS 1D



SDS 2D

Biopolymer materials



Bone graft

Bone fixation

Incorporation of other materials, eg: HA

Biopolymer materials

Membranes, coatings, fibrous assemblies

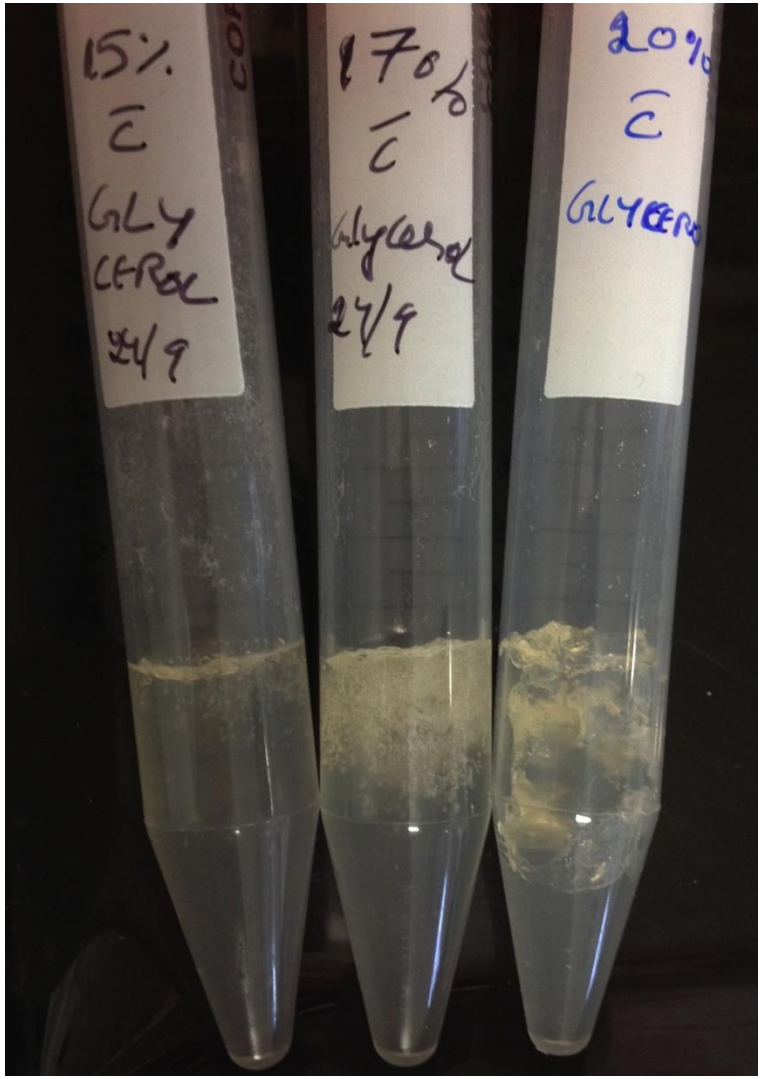
Biopolymer materials

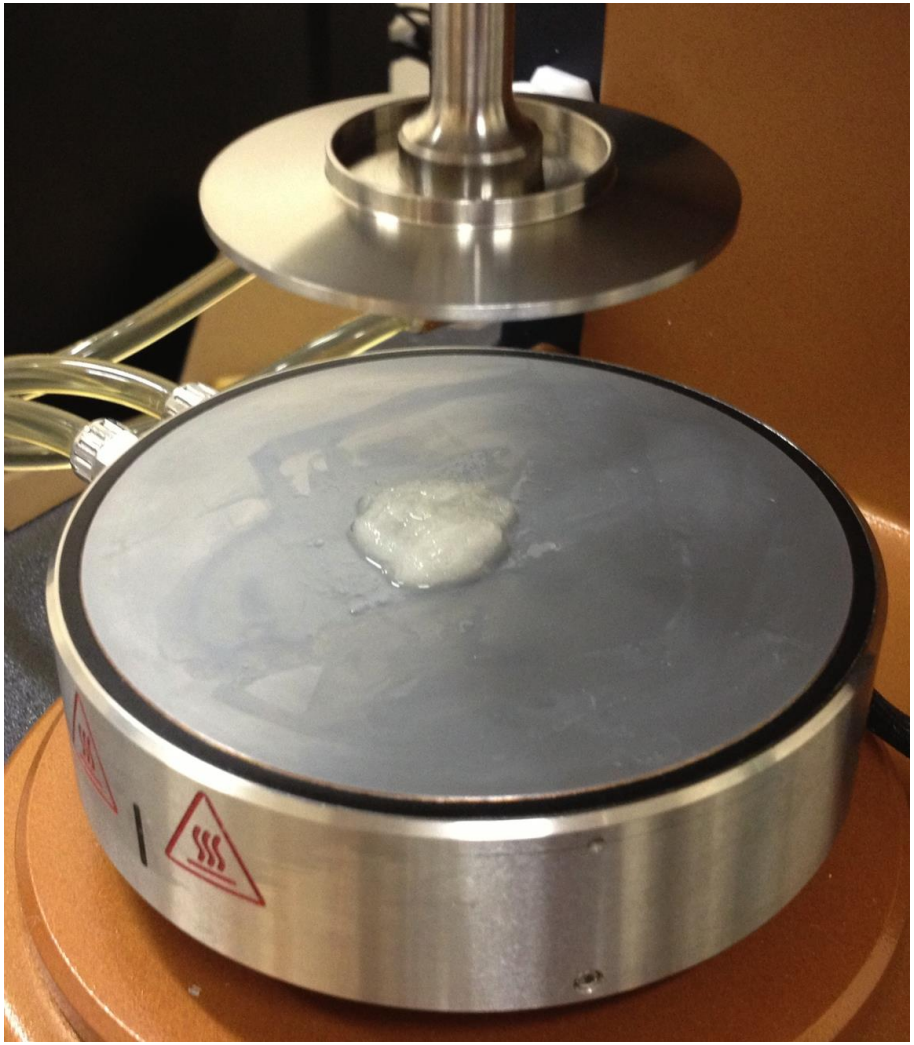
Soluble powder

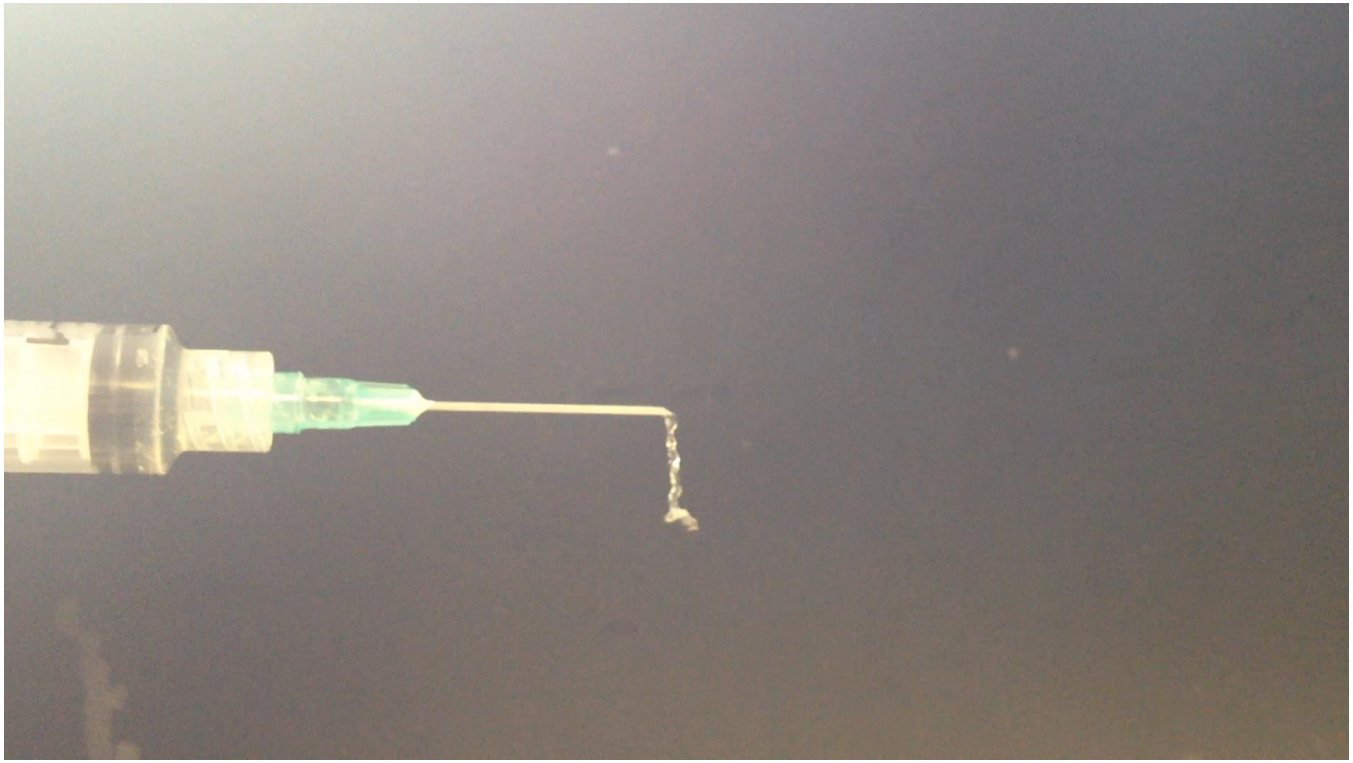
Gel forming liquid

Hydrogels

Keratin hydrogel



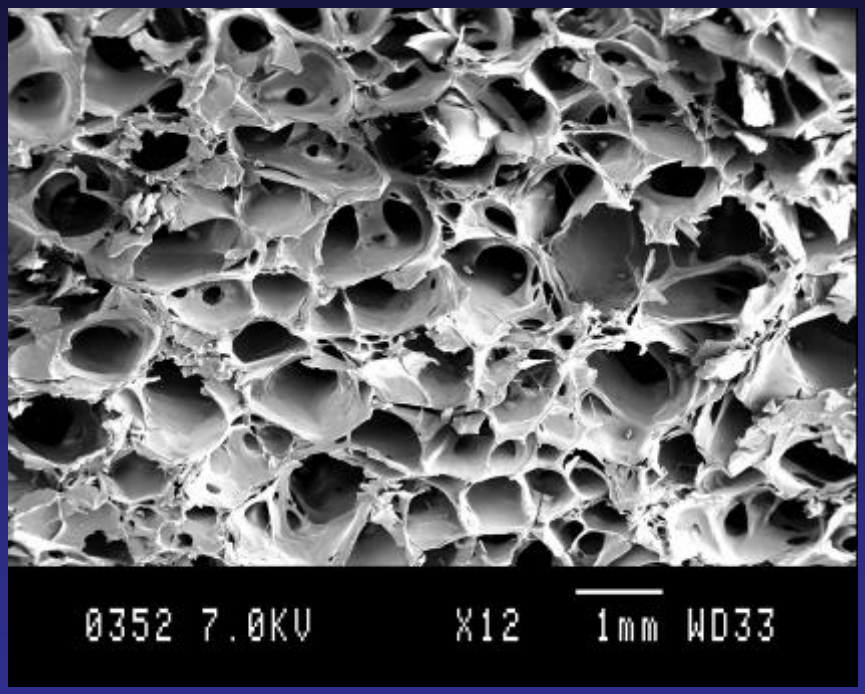


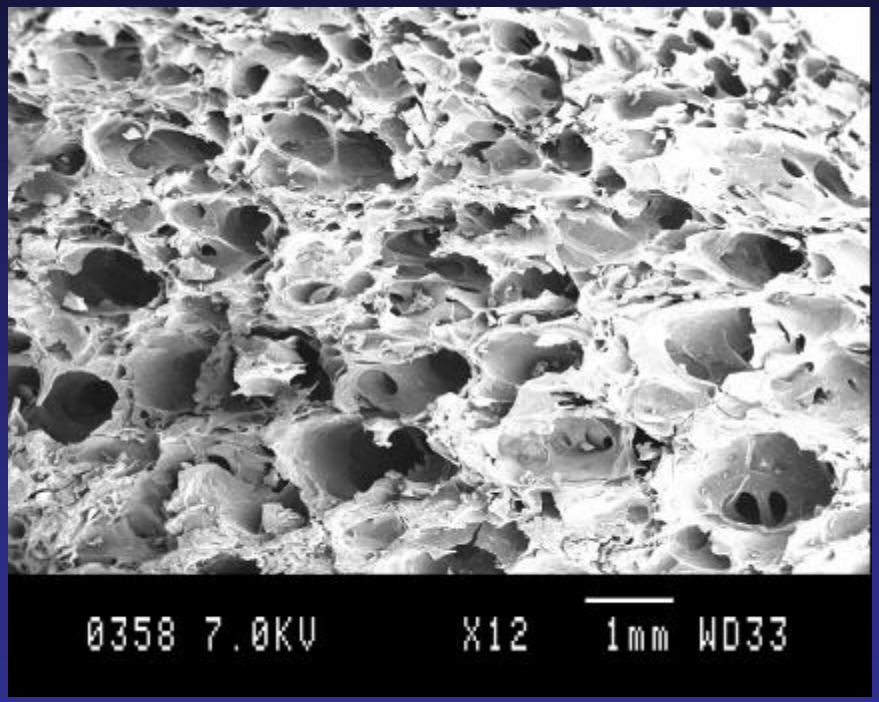


Control of porous structure

Leaching of porogens

Control of porous structure



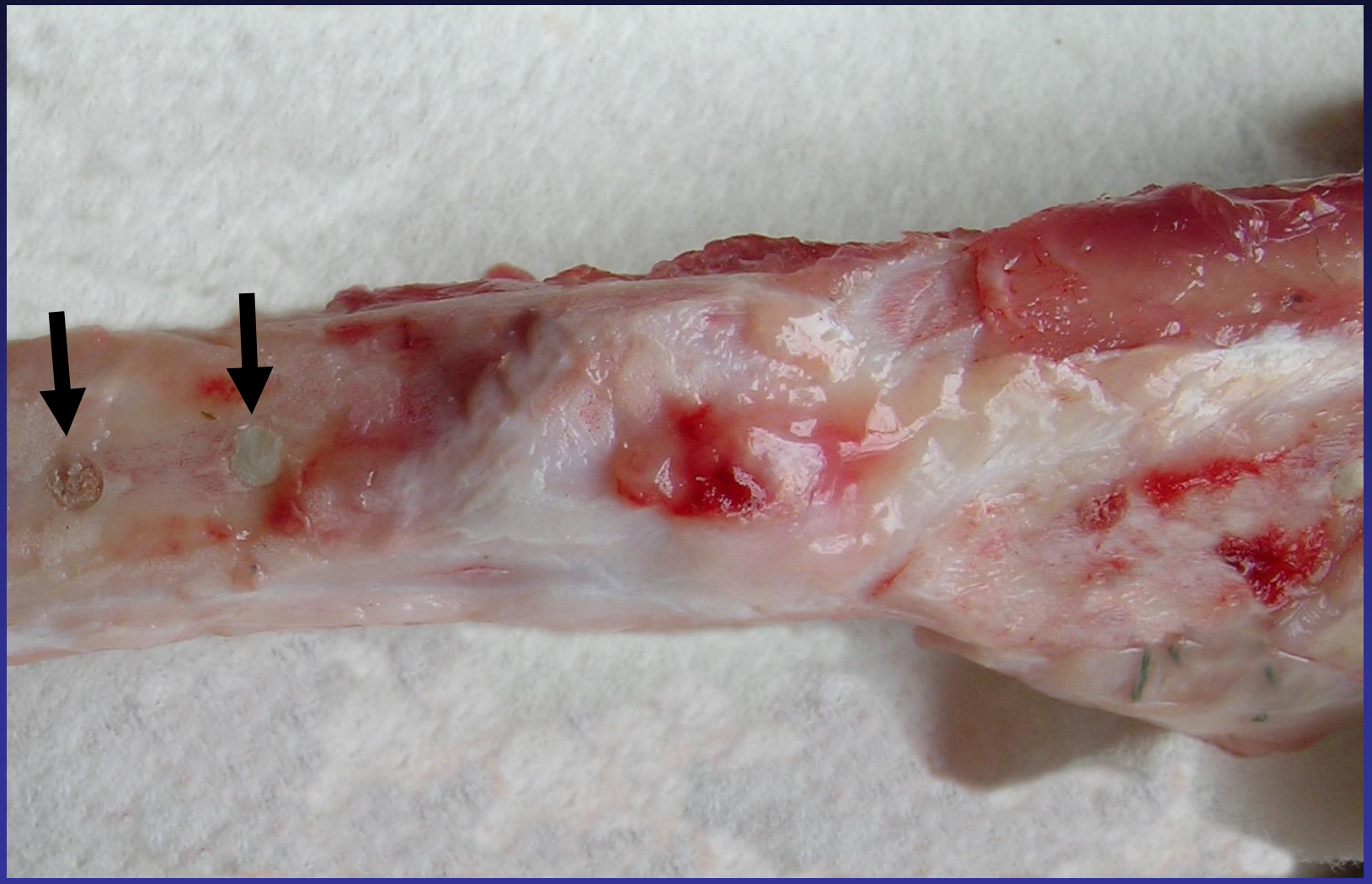


Keratin HA pore structure

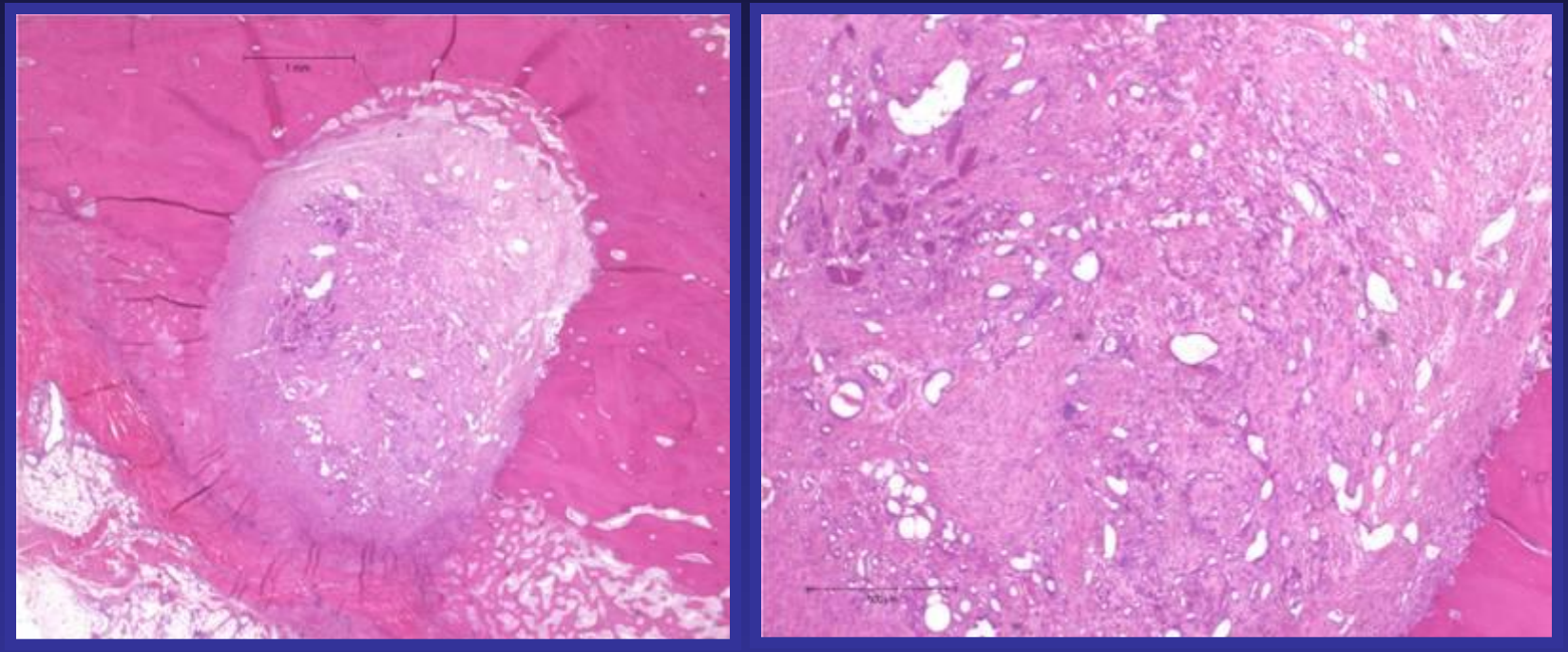
	KERATIN HA	CANCELLOUS BONE*
% density	29	20-25
Cell size / intertrabecular spaces (µm)	100-500	200-500

* Schenk, Biology of Fracture Repair

In Vivo Histomorphological Results

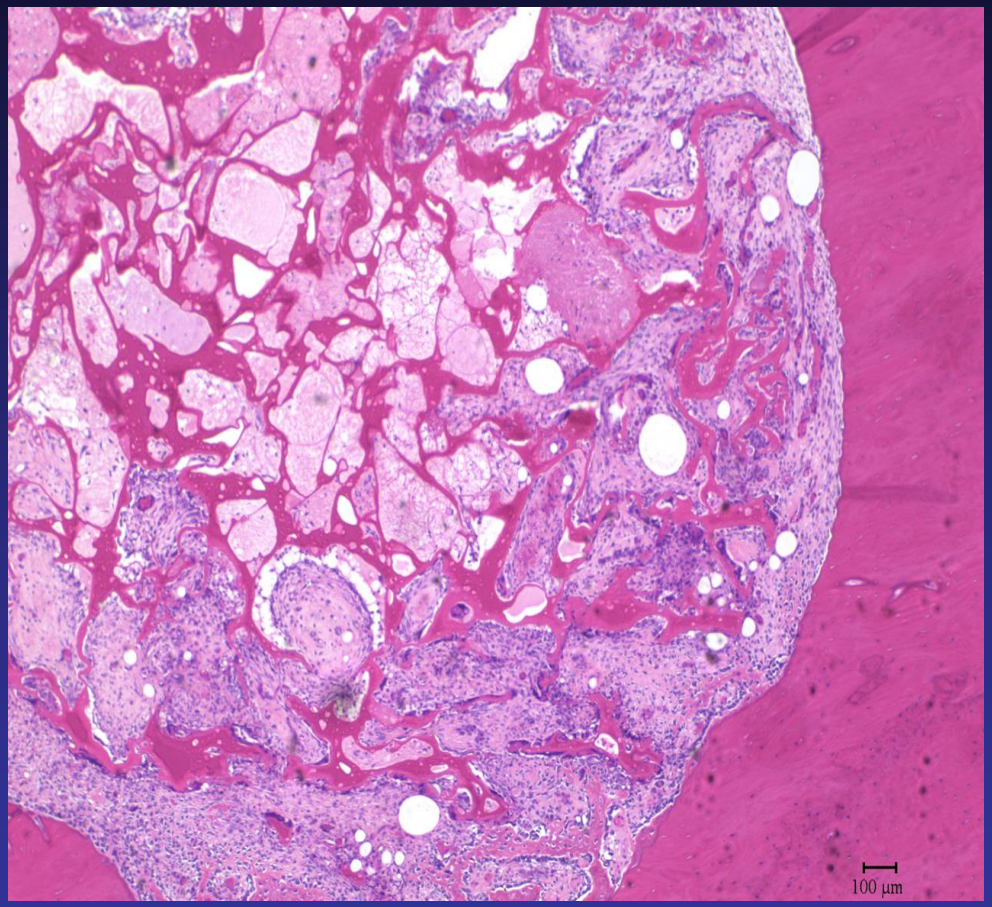


Results - Biological response



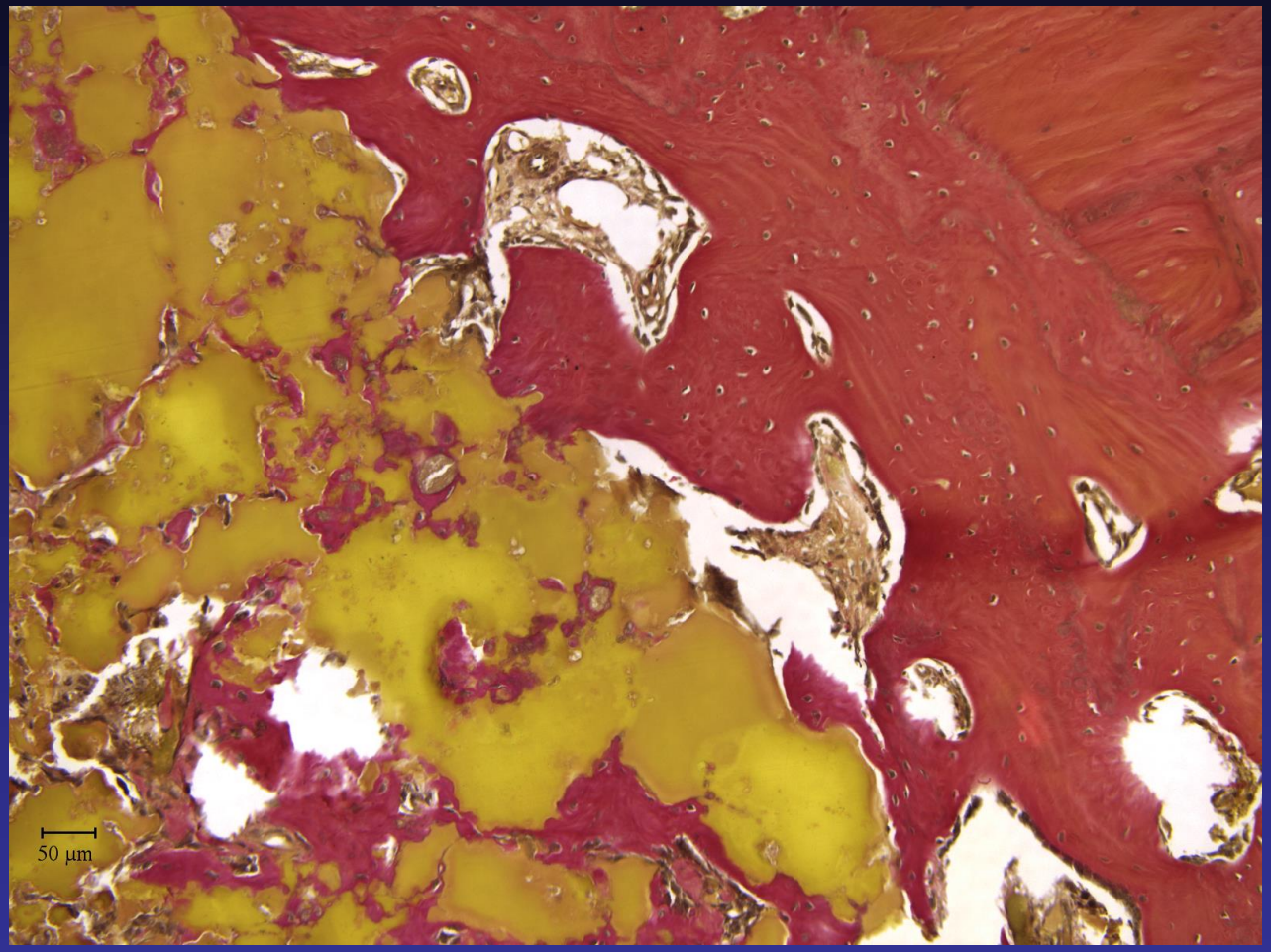
Collagen implant 3 weeks

Results - Biological response



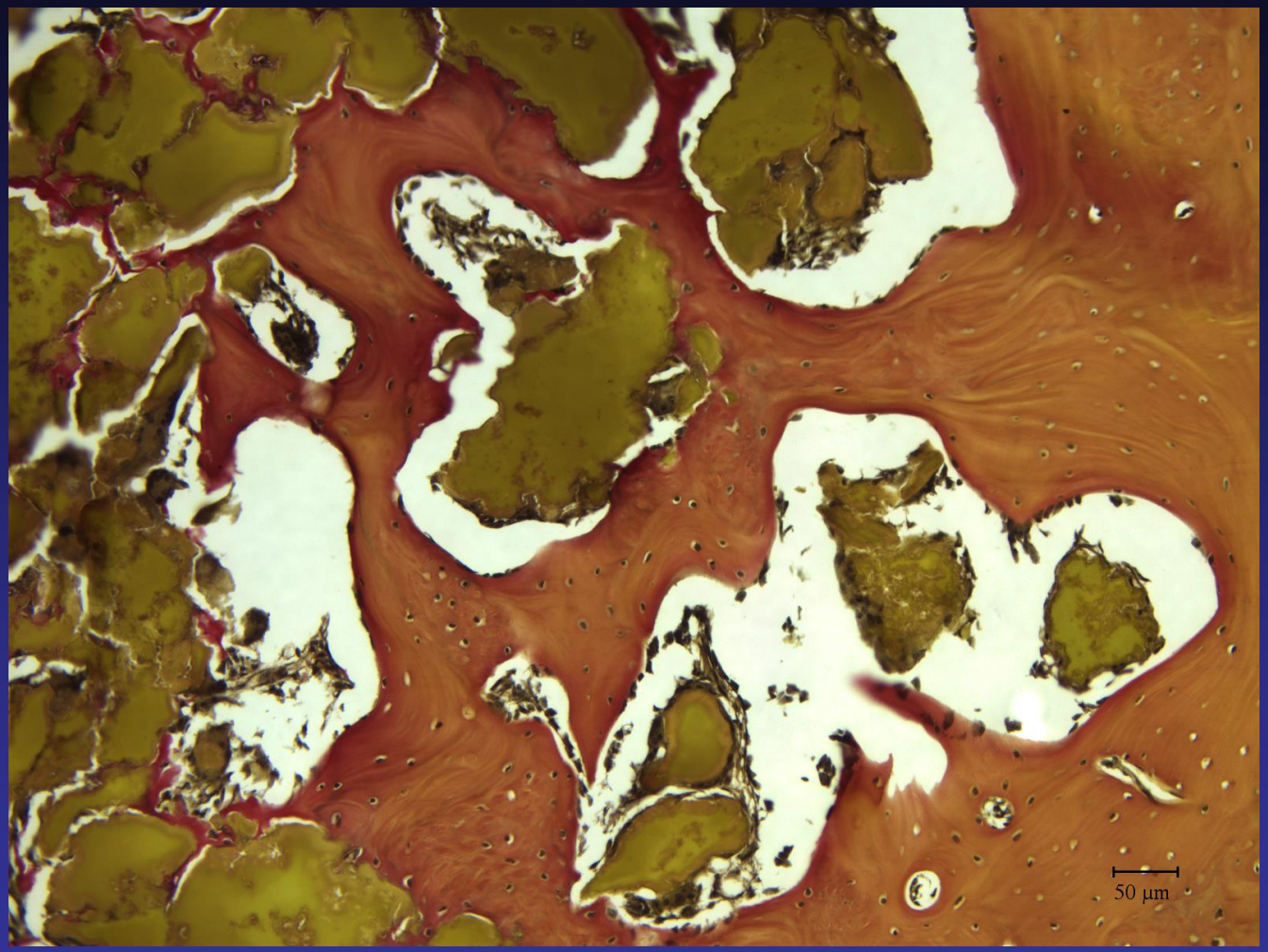
Porous keratin-4%HA implant 3 weeks

Results - Biological response



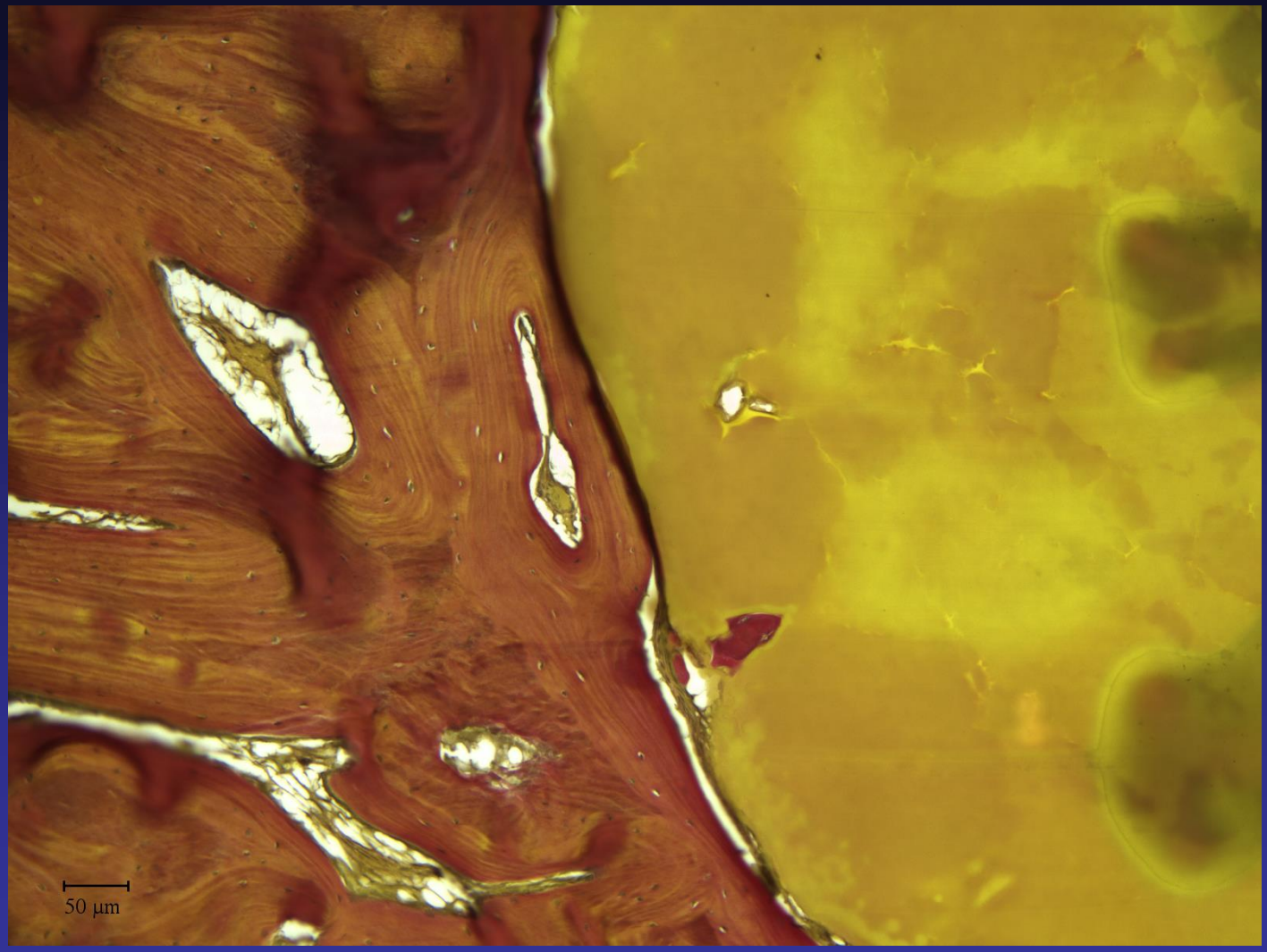
Porous keratin-4%HA implant 8 weeks

Results - Biological response



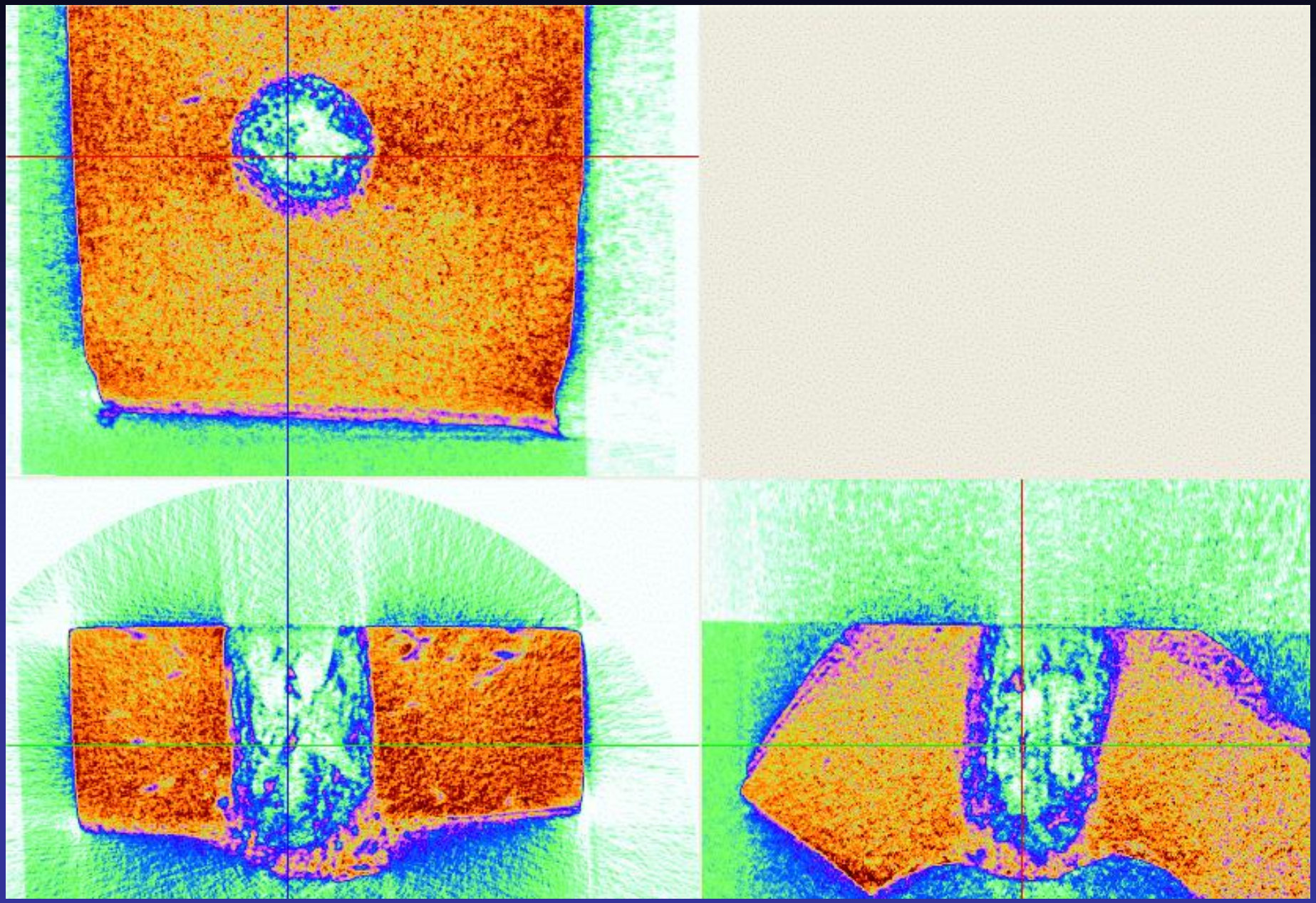
Porous keratin-4%HA implant 12 weeks

Results - Biological response

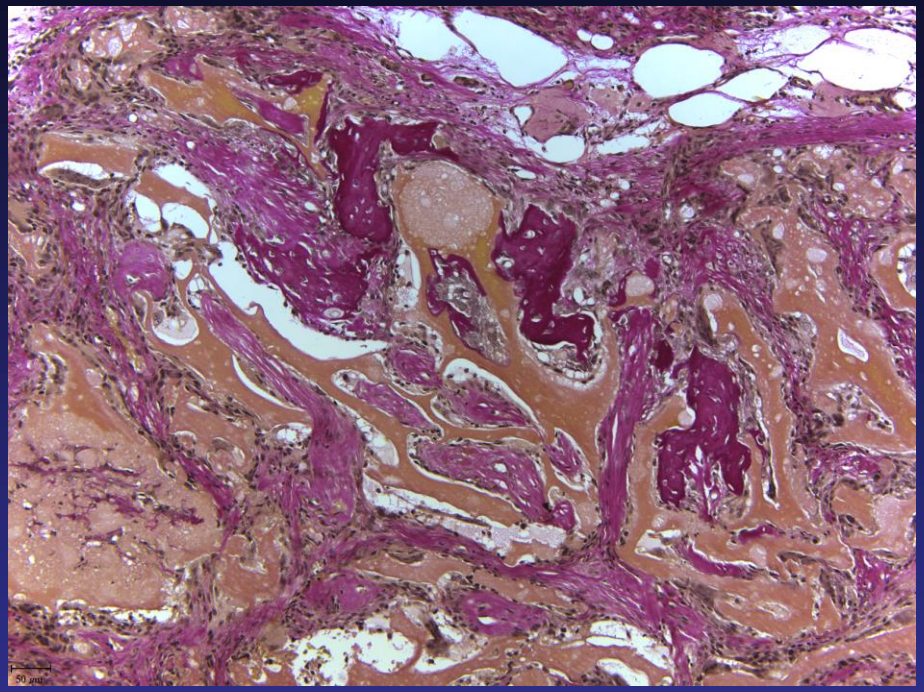


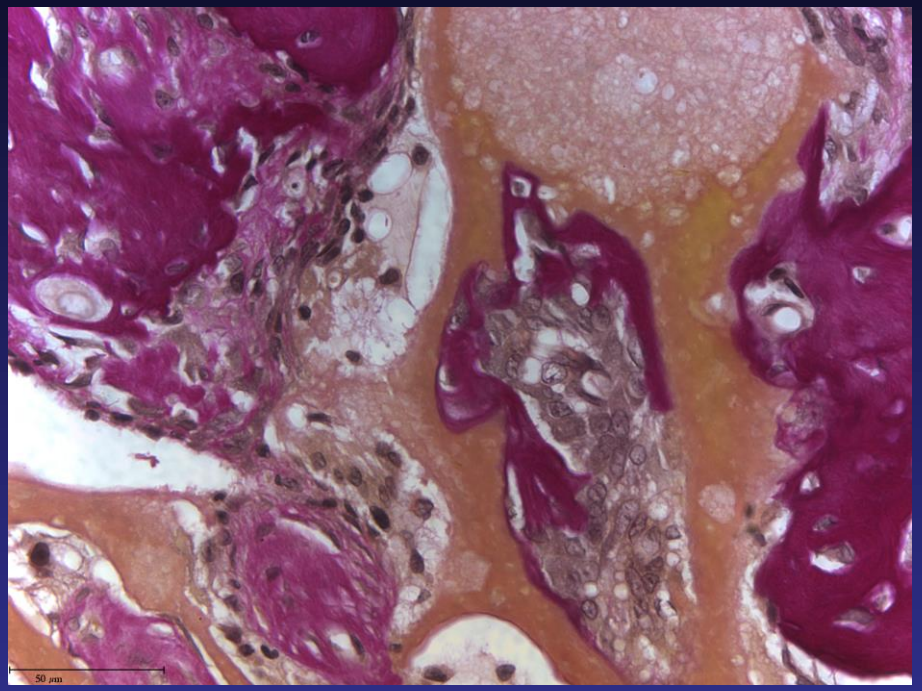
Dense keratin implant 12 weeks

Keratin HA/ Week 4/ Micro-CT



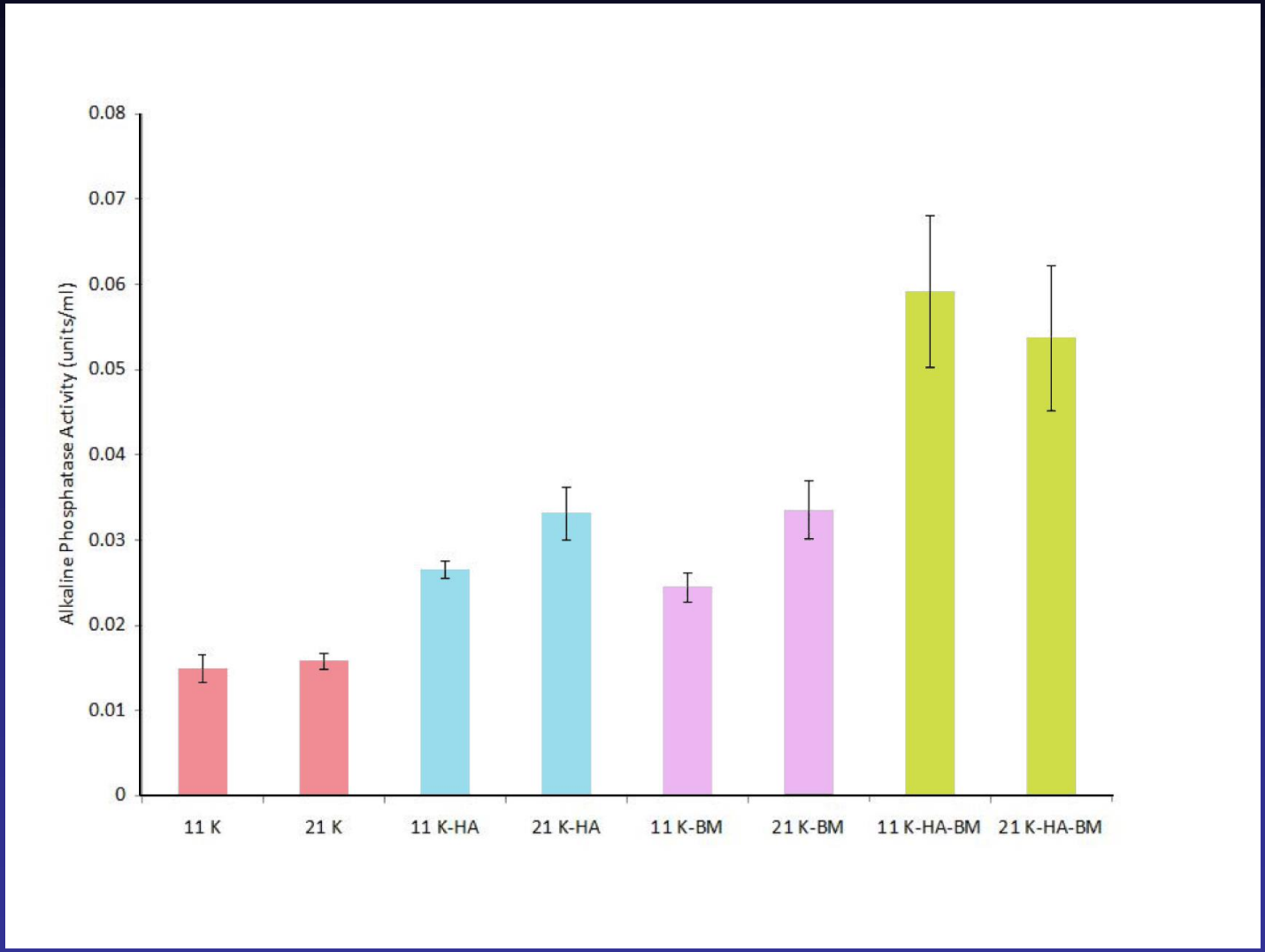
Osteoinduction





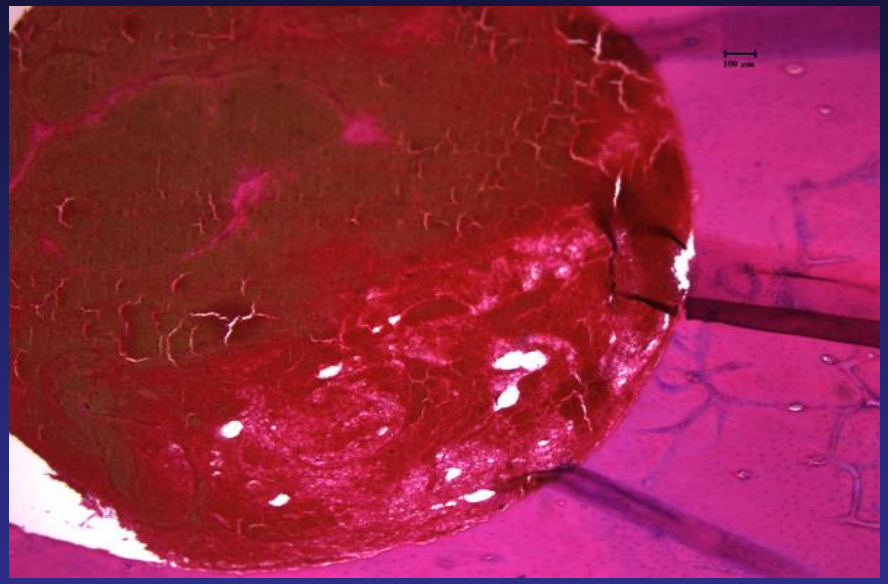
- KP/HA/BM 21 days sub-muscularly in Lewis rats
- Some cells show tendency towards osteoblastic differentiation & widespread osteoid may have formed with increased implantation times.

Osteoinduction

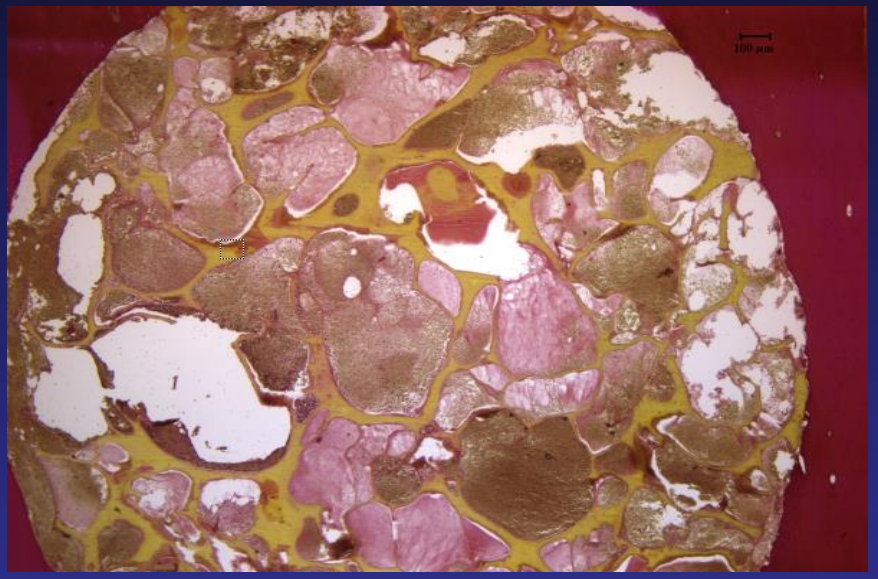


- KP/HA/BM 21 days sub-muscularly in Lewis rats

Week 1 (VG)

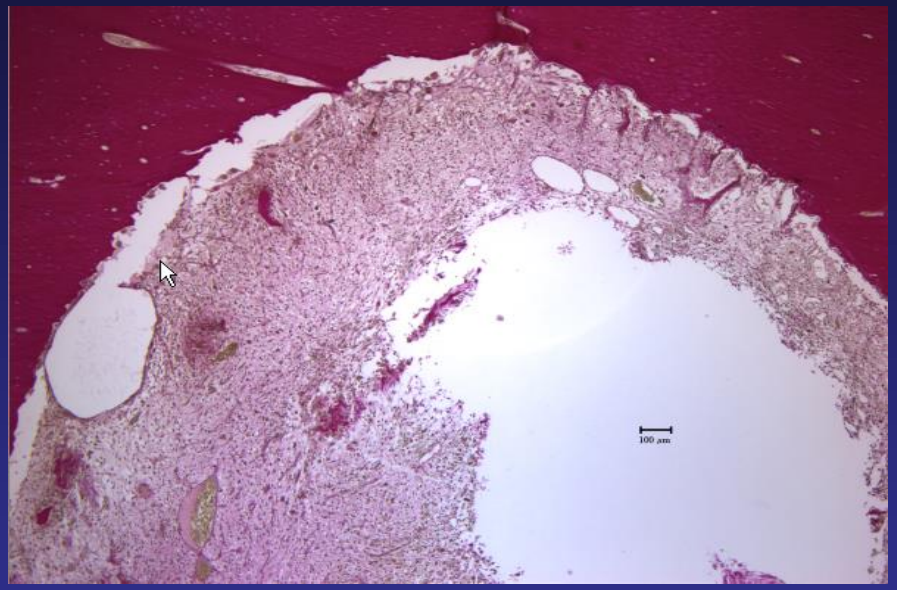


Sham

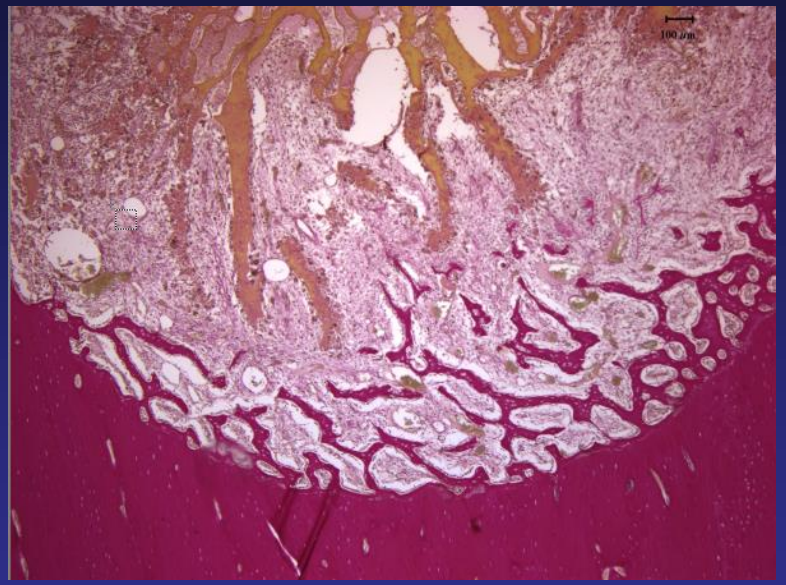


Keratin/HA

Week 2 (VG)

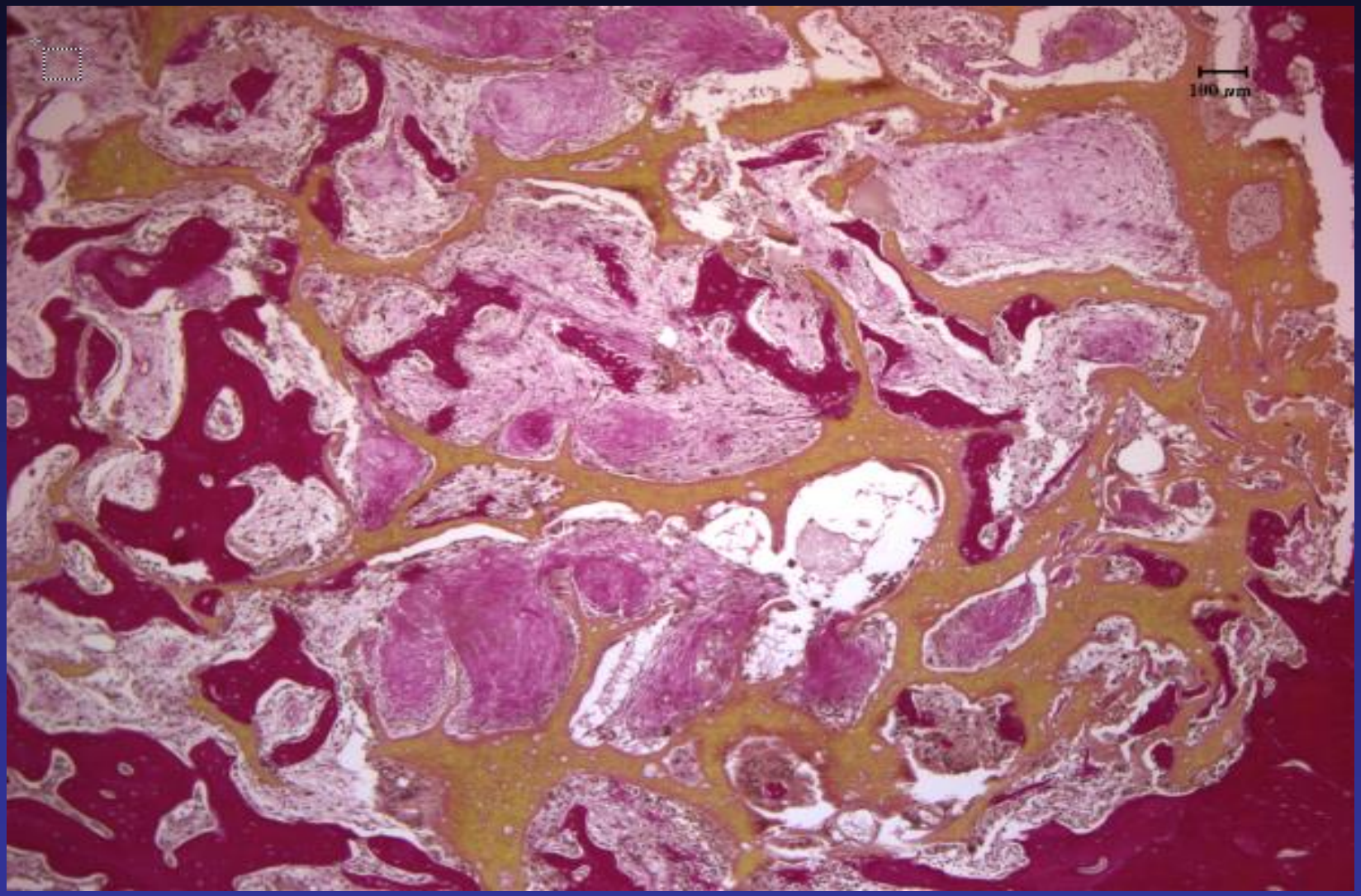


Collagen

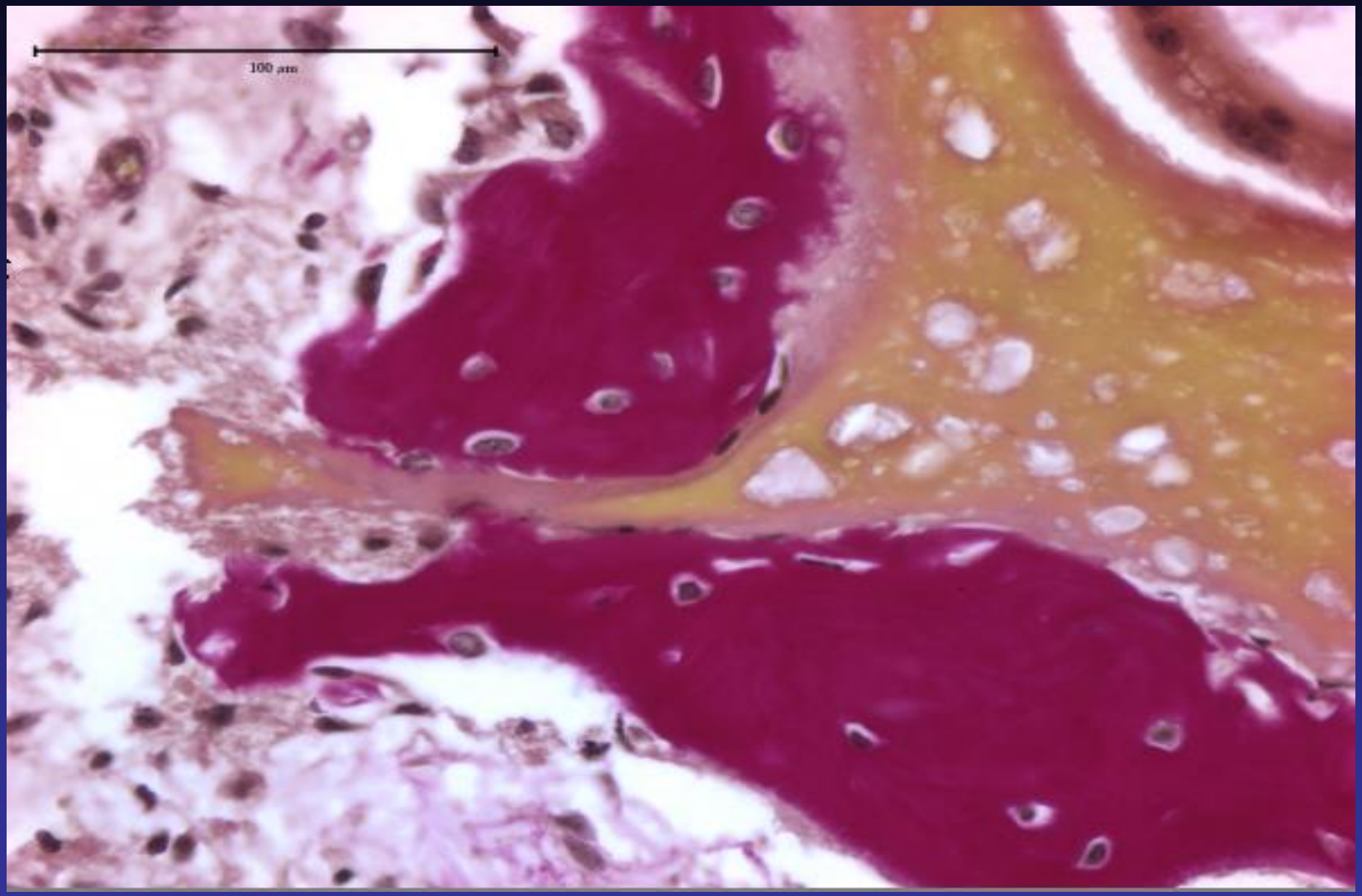


Keratin-40%HA

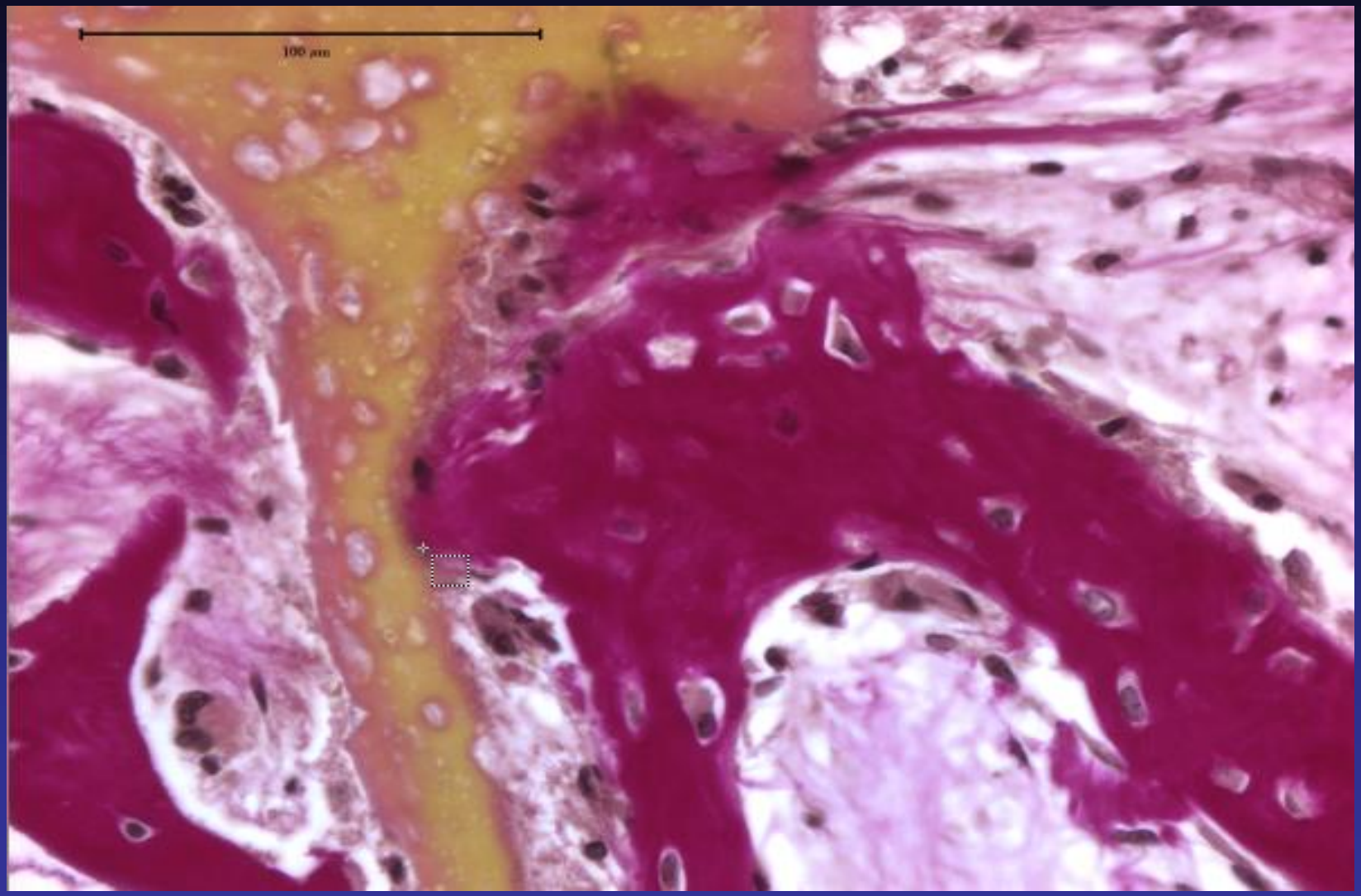
Trial 3: Keratin/HA Week 4 VG (x4)



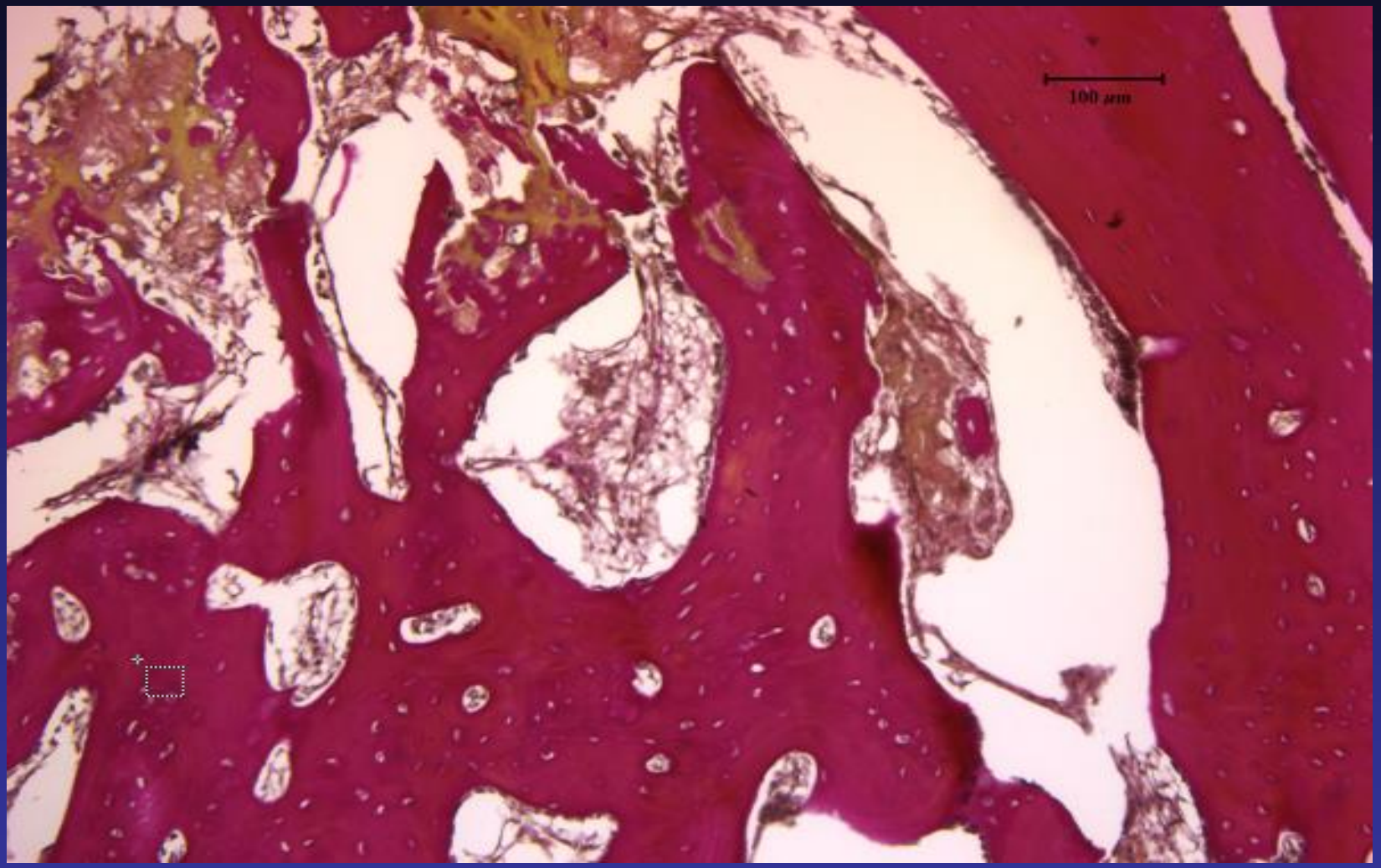
Trial 3: Keratin/HA Week 4 VG (x40)



Trial 3: Keratin/HA Week 4 VG (x40)



Trial 3: Keratin/HA Week 8 VG (x10)



Unique Properties of Keratin/HA

- Scaffold for cells and protein binding
- Calcifiable, biodegradable matrix
- Biocompatible
- Non-antigenic
- Stimulates osteoconduction, osteogenesis, and possibly osteoinduction

Patents

- Kelly, R.J., and Roddick-Lanzilotta, A.D. (Wool Research Organisation of New Zealand) and Dias, G.J., and Peplow, P.V. (University of Otago) Orthopaedic materials derived from keratin. No. PCT/NZ03/00116, International patent No. 537602 (2004)
- United States patent “Orthopaedic materials derived from keratin”, US 7,297,342, Peplow P.V., Dias G.J., Roddick-Lanzilotta, A.D., Kelly, R.J. issued in November 2007.

- Dias, G. J., Mahoney, P., Swain, M., Kelly, R. J., Smith, R.A., Mohammad, A.A. Keratin-Hydroxyapatite Composites: Biocompatibility, Osseointegration, and Physical Properties in an Ovine Model. *Journal of Biomedical Materials Research Part A,* **95A:** 1084-1095, 2010.

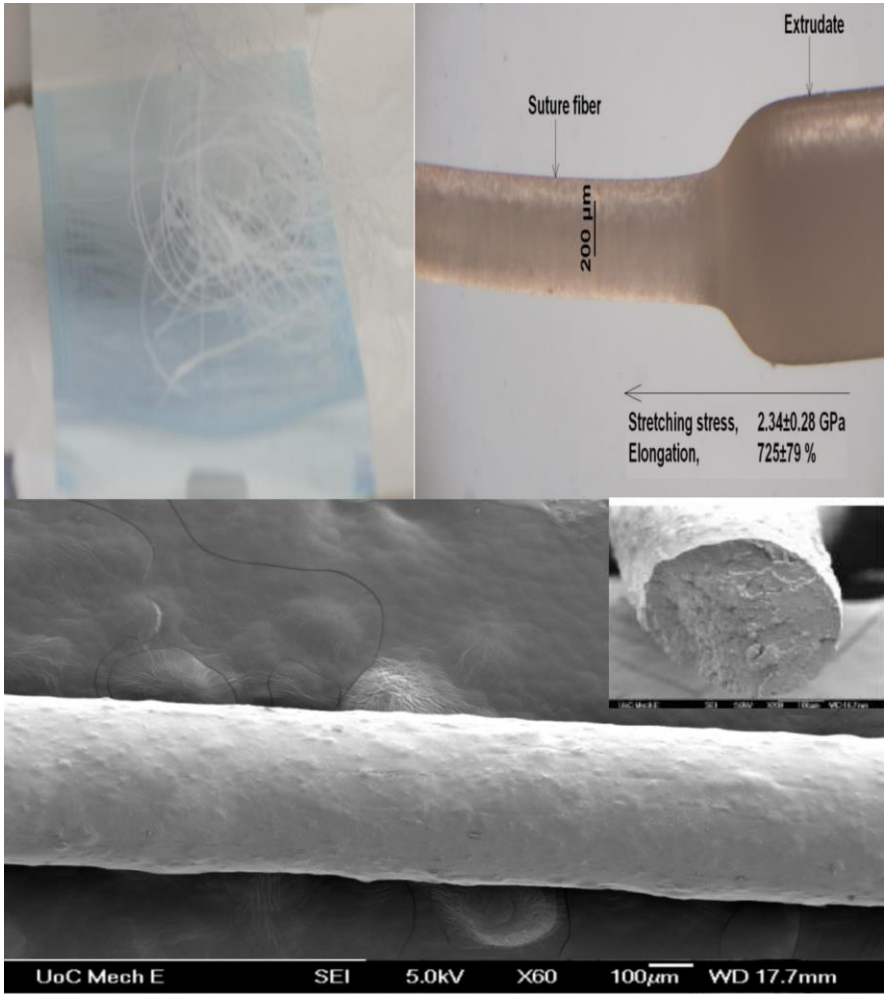
- Dias, G. J., Peplow, P. V., McLaughlin, A., Teixeira, F., & Kelly, R. J. (2010). Biocompatibility and osseointegration of reconstituted keratin in an ovine model. *Journal of Biomedical Materials Research Part A,* **92A:** (2), 513-520.

- Peplow, P. V., Dias, G. J., Teixeira, F., & Kelly, R. J. (2009) Tissue reaction to matrices of reconstituted keratin polymer implanted subcutaneously in sheep. *Journal of Biomedical Materials Research Part A,* **89A**: (1), 255-265.

- Peplow P. V. and Dias G. J. A study of the relationship between mass and physical strength of keratin bars in vivo. *Journal of Materials Science: Materials in Medicine*, 2004; 15:1217-1229

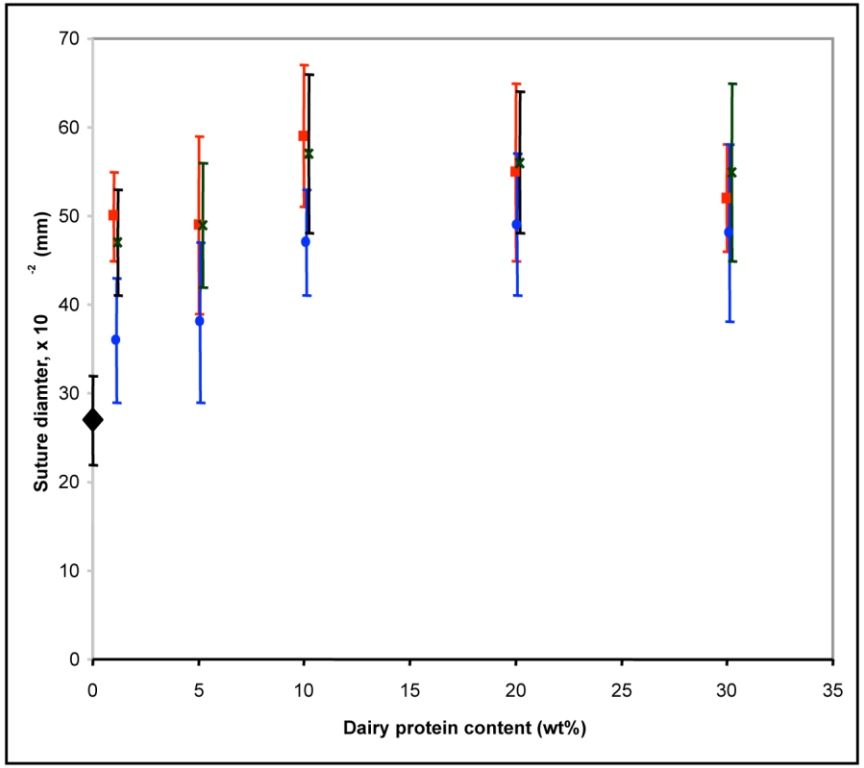
Novel hybrid resorbable sutures

- A novel processing method was established to produce dairy protein based bio-absorbable hybrid medical suture, which retain positive biological properties of dairy proteins.
- The methods involved in processing are simple and similar to melt extrusion fibre manufacturing.
- This processing methodology uses moderate mixing temperature **without any chemical agents, hence will not affect the physiological or biological benefits of proteins.**
- The hybrid suture demonstrated **unique characteristics of surface and matrix morphologies.**



Suture diameters

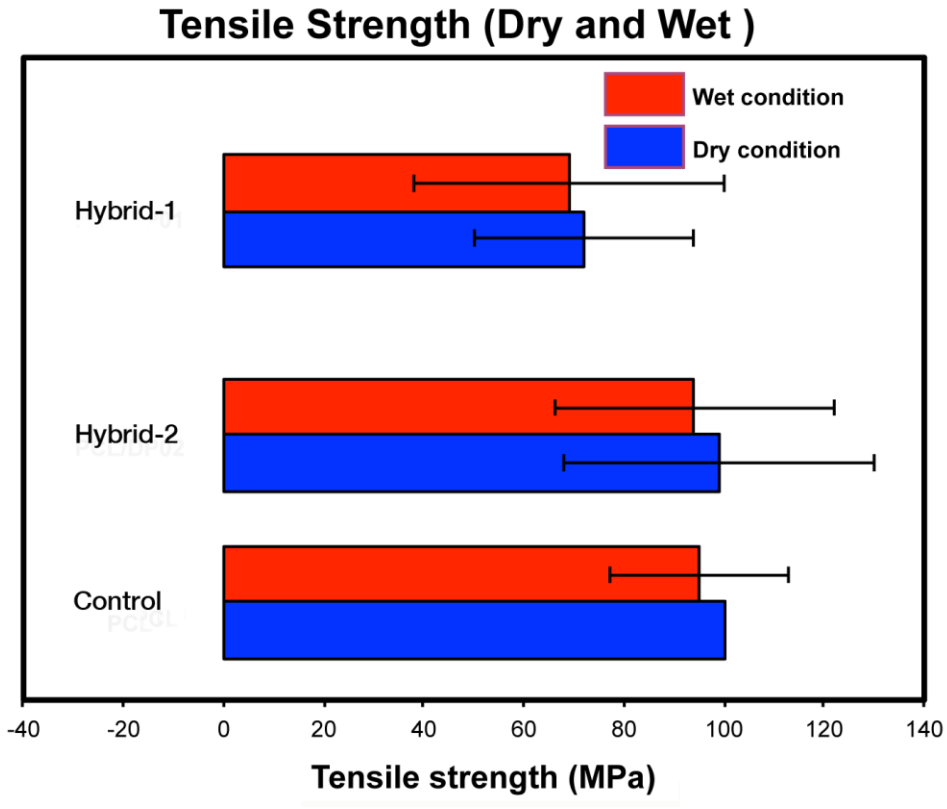
- The manufacturing process can produce variable suture diameters.
- Approximately30-50μm diameter of mono-filament suture was produced for this investigation.
- Processing methodology demonstrated that this melt-extrusion process can also be applied to produce multi-filament sutures.



Mechanical performance of sutures

- Hybrid sutures (DP01 & DP02) comprising desirable proportions of dairy proteins exhibited good mechanical properties similar to the control (PCL based biodegradable suture) in dry and wet conditions.

- Tensile strength (in dry & wet conditions) results demonstrated that the hybrid suture having suitable mechanical characteristics that is desirable in a medical suture.

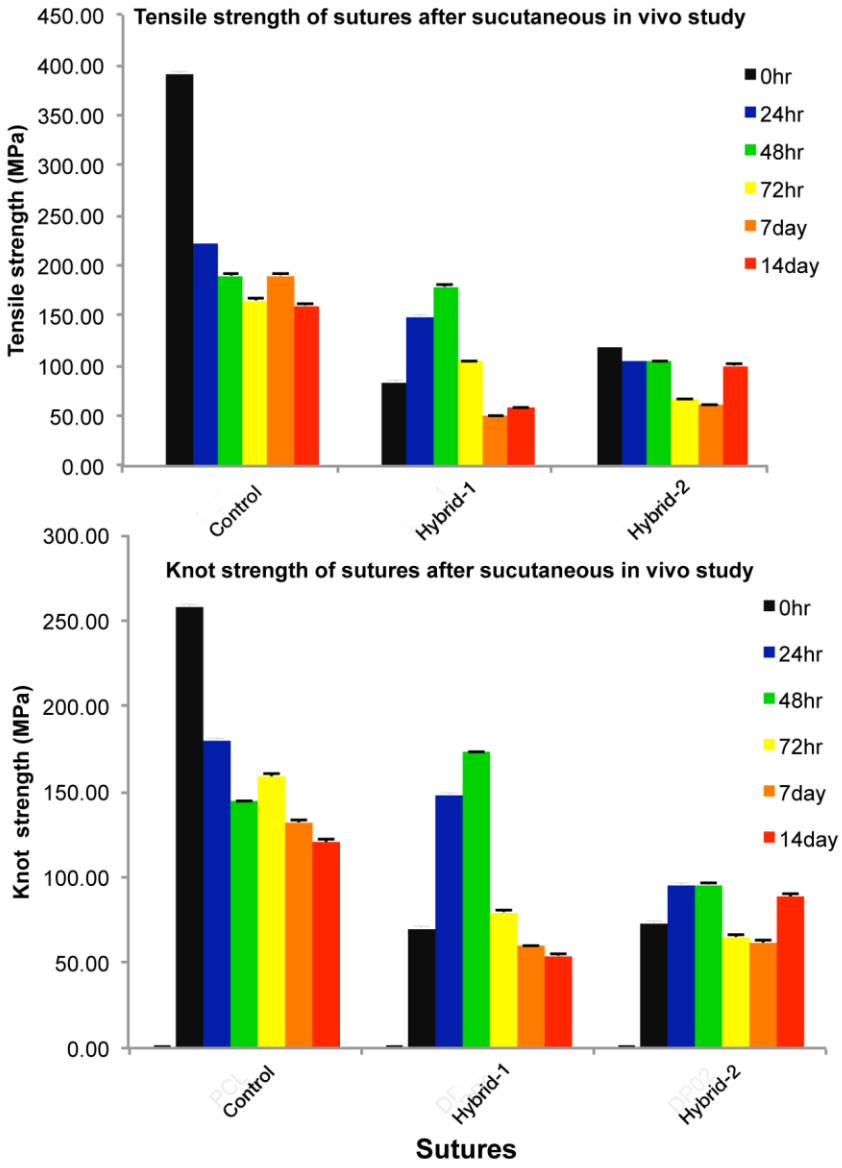


In vitro/in vivo results

- The tensile strength (TS) and knot strength (KS) of hybrid sutures in particular DP02 suture remained almost unchanged after *in vivo* subcutaneous implantation (in rats) for 14 days. In contrast, these properties of the control suture (PCL based commercial) decreased with time.

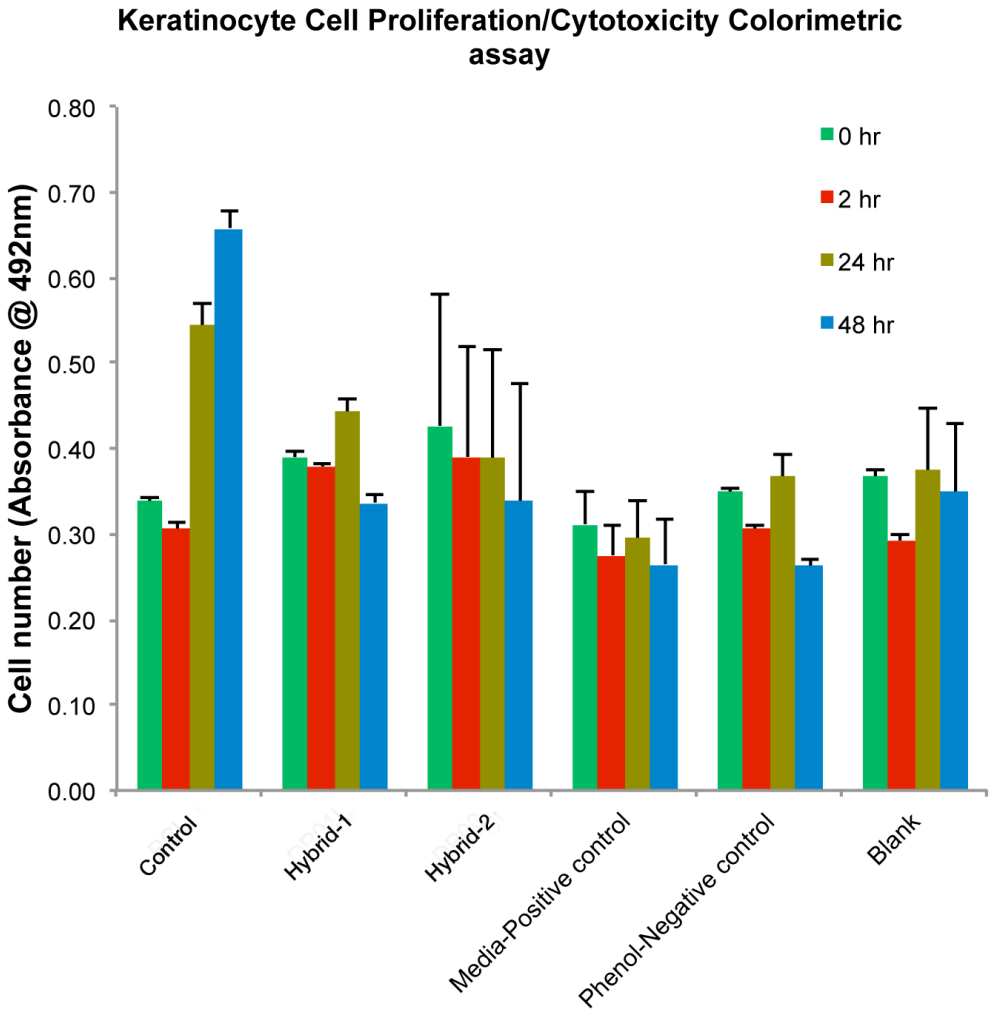
- Significant drop in KS of PCL suture which may be of concern in clinical applications.

- Hybrid sutures constantly retained TS and KS (DP02 suture) during subcutaneous *in vivo* implantation at the different time-points. These results indicate that hybrid sutures possibly integrating with skin tissues leading to enhancement of TS & KS *in vivo* at 14 days.

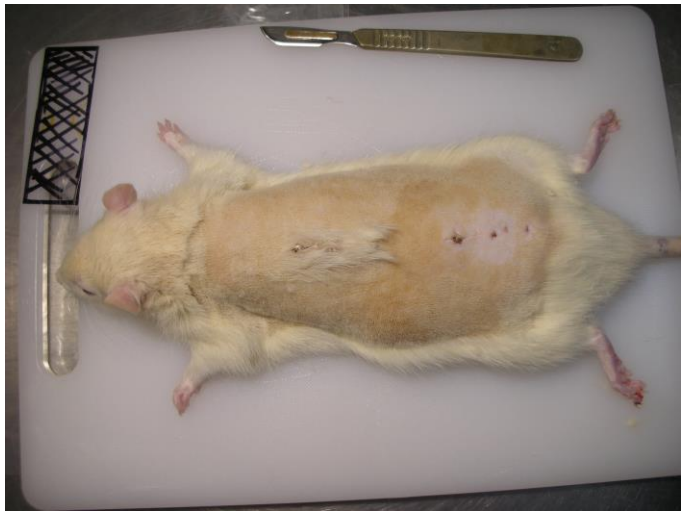


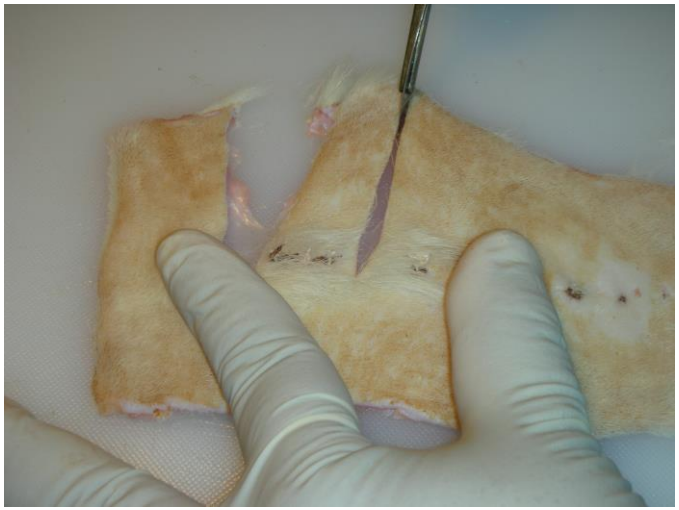
In vitro/in vivo results

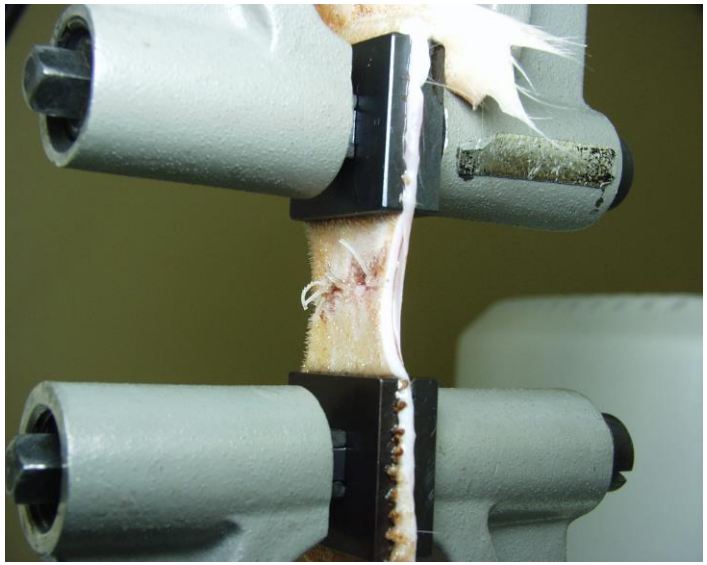
- The hybrid sutures demonstrated biocompatibility and satisfactory keratinocyte (skin cell) proliferation, comparable to PCL.
- The following skin closure *in vivo* investigation was based on these results.

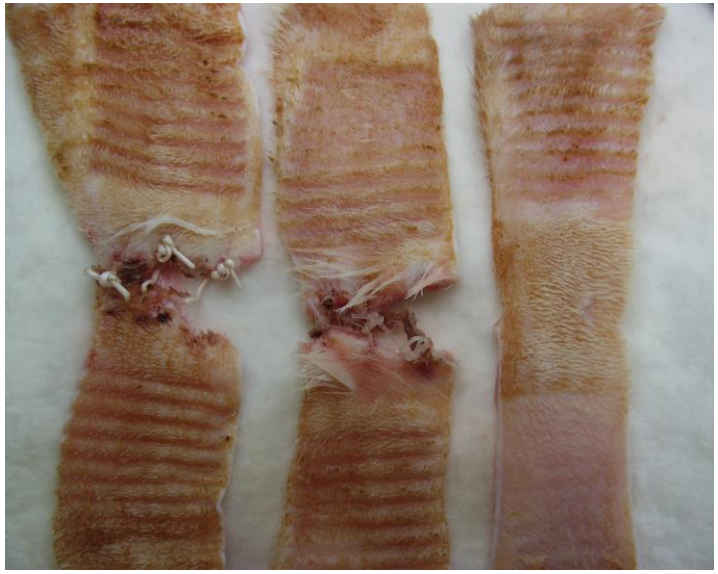


SKIN/WOUND CLOSING STUDY *IN VIVO*



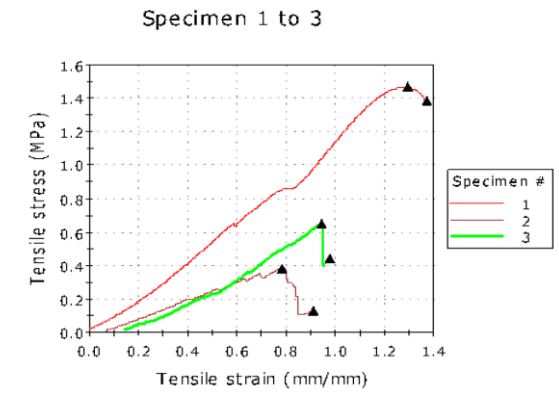




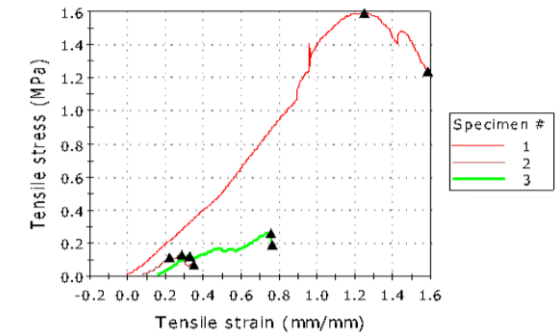


SKIN/WOUND CLOSING STUDY *IN VIVO*

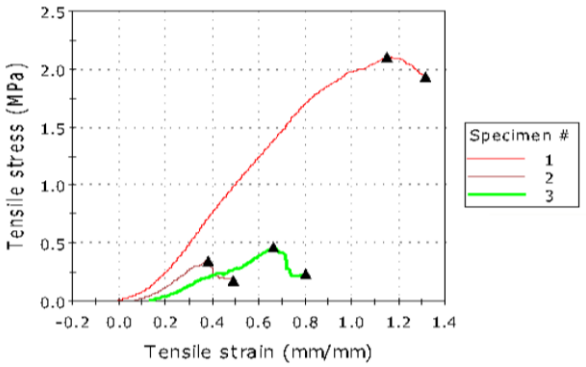
- Skin/wound closing study was conducted in the Lewis rat.
- The wound strengths were approximately 30% greater at the 2 experimental suture wound closing sites compared to the PCL control at 7 days.
- This important finding illustrates that the hybrid suture comprising dairy protein are fully bioabsobable and enhances wound healing leading to significant increase in wound strength compared to PCL based suture.
- The skin healing/closing results of hybrid sutures *in-vitro* and *in-vivo* (animal model) studies reveal that sutures comprising dairy protein demonstrating **remarkable skin wound healing/closing performance.**



7 days



14 days

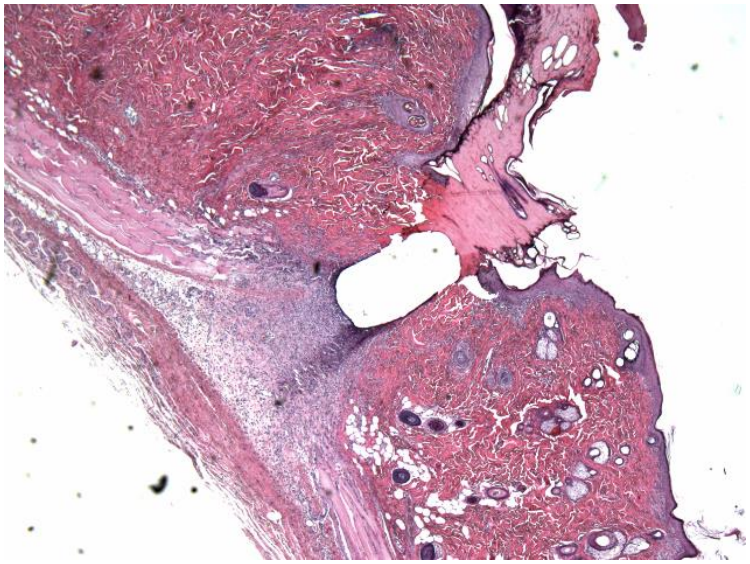


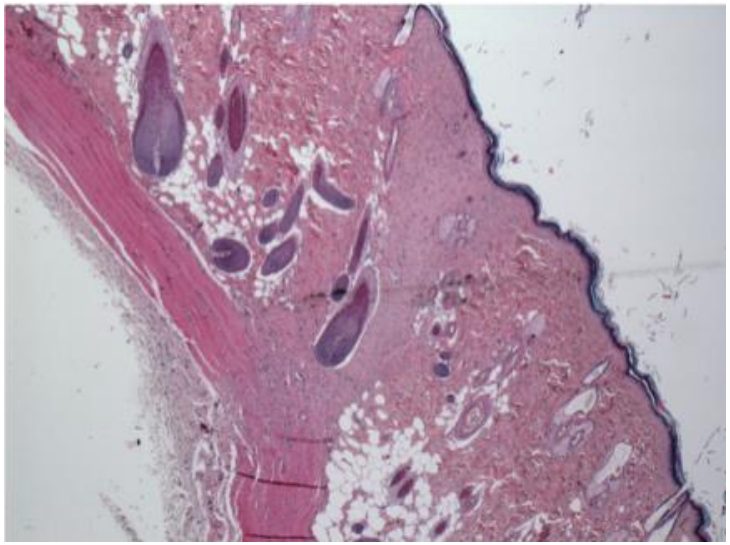
28 days

1- normal skin, 2-control suture site, 3-hybrid suture site

SKIN/WOUND CLOSING STUDY *IN VIVO*

	Control	Material A	Material B
Day 7	549.95 $\mu m^2/\mu m$	578.29 $\mu m^2/\mu m$	661.68 $\mu m^2/\mu m$
Day 14	1249.04 $\mu m^2/\mu m$	722.26 $\mu m^2/\mu m$	422.47 $\mu m^2/\mu m$
Day 28	665.97 $\mu m^2/\mu m$	510.76 $\mu m^2/\mu m$	496.92 $\mu m^2/\mu m$





Competitive Advantages with our technology

- The current medical sutures (both resorbable and non-resorbable) in the market **provide mechanical support to lesions in tissues**. **These do not claim any physiological and biological benefits.**
- The hybrid sutures produced by incorporating dairy protein based biomaterials into biocompatible polymer demonstrate **significantly improved healing performance and tissue-integration, with desirable mechanical properties**. These balanced attributes demonstrates significant potential for creating new generation of medical sutures.
- However, suture production process may require further improvement to achieve higher **mechanical properties**, and **to fuse needle onto the suture (eye-less needle)**.
- Further small and large animal trials are also required prior to pre-clinical trials.
- While exact manufacturing cost is unknown at this stage; it is envisaged that the cost would be approximately 10% higher than the current PCL based sutures.

Acknowledgements

Funding:
Otago Research Grants (3)
Otago/AgResearch Grant
Keratec Ltd (New Zealand)
Keraplast Technologies Ltd (USA)

- Collaborators:
- Dr. Rob Kelly
- Dr. Azam Ali
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