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8 PUBLICATIONS MADE BASED ON THIS RESEARCH

Anyango, J.O., Taylor, J., Taylor, J.R.N., 2011. Improvement in water stability and other related functional properties of thin cast kafirin protein films. *Journal of Agricultural and Food Chemistry* 59, 12674–12682.

Anyango, J.O., Duneas, N., Taylor, J.R.N., Taylor, J., 2012. Physico-chemical modification of kafirin microparticles and their ability to bind bone morphogenetic protein-2 (BMP-2), for application as a biomaterial. *Journal of Agricultural and Food Chemistry* 60, 8419–8426.

9 ANNEX

Annex 1: Approval document

Annex 2: Idexx Laboratories Report



ANNEX 1: APPROVAL DOCUMENTS



UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA



UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

ANIMAL USE AND CARE COMMITTEE

Private Bag X04
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Tel +27 12 529 8434 / Fax +27 12 529 8300
e-mail: aucc@up.ac.za

Ref: H016-11

19 April 2011

Prof JRN Taylor
Department of Food Science
University of Pretoria
(john.taylor@up.ac.za)

Dear Prof Taylor

H016-11 : Subcutaneous bioassay of novel osteoconductive protein microstructure for alternative autograft bone transplant scaffold (J Anyango)

The application for ethical approval, dated on 31 March 2011 was approved, by the Animal Use and Care Committee at its meeting held on 18 April 2011.

Kind regards

Elmarie Mostert

AUCC Coordinator

Copy Prof V Naidoo

J Anyango



ANIMAL USE AND CARE COMMITTEE

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Ref: H016-11 (Amendment 1)

1 February 2012

Prof JRN Taylor
Department of Food Science
University of Pretoria
(john.taylor@up.ac.za)

Dear Prof Taylor

H016-11 (Amendment 1) : Subcutaneous bioassay of novel osteoconductive protein microstructure for alternative autograft bone transplant scaffold (J Anyango)

The amendment to the application for ethical approval, dated 19 December 2011 was approved by the Animal Use and Committee at its meeting held on 30 January 2012.

Kind regards

Elmarie Mostert

AUCC Coordinator

Copy Prof V Naidoo

J Anyango



ANNEX 2: IDEXX LABORATORIES REPORT

UNIVERSITY OF PRETORIA
BIOMEDICAL RESEARCH CENTRE
PRIVATE BAG X04
ONDERSTEPSPOORT
0110

2012/04/04

For attention: Prof Vinny Naidoo and Ilse Janse van Rensburg

**STUDY H16/11: SUBCUTANEOUS BIOASSAY OF NOVAL OSTEOCONDUCTIVE
PROTEIN MICROSTRUCTURE FOR ALTERNATIVE AUTOGRAFT BONE
TRANSPLANT SCAFFOLD: IDEXX REF 2000/12 AND 2892/12**

INTRODUCTION:

We have received two groups of Sprague-Dawley rats, namely group 1 with our reference 2000/12 on the 21st of February 2012, and a second group with our reference 2892/12 on the 13th of March 2012. All of these rats had four implantation sites on the dorsal body wall, which we allocated the numbering system A1 and A2 on the left hand side anterior and posterior respectively, and on the right hand side B1 and B2 anterior and posterior respectively. The implantation sites were evaluated and measured and the findings are recorded in Table 1A and 1B of both these groups.

We were requested to evaluate the implant sites as well as the standard histology on organs with a special request to look at the bone morphogenesis of the implant sites and to record the findings as well as the digital photos of specific morphological findings. In the second group 2892/12 two of the implantation sites were collected by the researcher for additional testing and were not available for histopathology. Histopathology results of the implantation sites were tabulated and the findings recorded (Tables 2A and 2B for 21/2/12 and 13/3/12 respectively).

METHODOLOGY:

Necropsies were performed and the implantation sites were all collected separately in 10% buffered formalin as indicated above, while organs were collected for the standard histopathology profile in a separate formalin container.

After sufficient fixation (2 days) selected blocks of tissue as well as a cross section of the implantation site were cut and processed in an automated histological tissue processor according to our standard operating procedure for our Idexx laboratory (PTA-his-SOP-27). After overnight tissue processing, wax blocks were produced and histological sections of 6µm were cut on a microtome (PTA-his-SOP-30). The slides were then stained with Haematoxylin and Eosin staining in an automated histological stainer (PTA-his-SOP-25).

The macroscopical findings of the implantation sites for these two groups of rats are recorded in Table 1A and 1B.

Histological observations of the implantation sites for these two groups of rats are given in Table 2A and 2B.

MACROSCOPICAL FINDINGS OF IMPLANTATION SITES

UPBRC Ref: H16/11 Our Ref: 2000/12

Date received: 21/02/2012

<i>Macroscopical evaluation of implantation sites</i>				
<i>Rat no</i>	<i>UPBRC ref</i>	<i>Histo ref</i>	<i>Morphological Appearance</i>	<i>Size (mm)</i>
14	14/1	2000-14 A1/12	Cystic granuloma	20
	14/3	2000-14 A2/12	Small cystic lesion	15
	14/2	2000-14 B1/12	Cystic granuloma	20
	14/4	2000-14 B2/12	Round nodule	10
15	15/1	2000-15 A1/12	Hard flat implant	10
	15/3	2000-15 A2/12	Hard flat implant	10
	15/2	2000-15 B1/12	Hard flat implant	15
	15/4	2000-15 B2/12	Hard flat implant	20
16 F	16/1	2000-16F A1/12	Round flat nodule	18
	16/3	2000-16F A2/12	Cystic granuloma	22
	16/2	2000-16F B1/12	Cystic granuloma implant	10
	16/4	2000-16F B2/12	Cystic granuloma	25
16 M	16/1	2000-16M A1/12	Granulomatous nodule	15
	16/3	2000-16M A2/12	Flat hard implant	18
	16/2	2000-16M B1/12	Flat nodule	12
	16/4	2000-16M B2/12	Round granulomatous nodule	15
17	17/1	2000-17 A1/12	Cystic granuloma	20
	17/3	2000-17 A2/12	Flat hard implant	15
	17/2	2000-17 B1/12	Cystic nodule	15
	17/4	2000-17 B2/12	Hard flat implant	15
18	18/1	2000-18 A1/12	Hard flat implant	10
	18/3	2000-18 A2/12	Hard flat implant	15
	18/2	2000-18 B1/12	Hard flat implant	10
	18/4	2000-18 B2/12	Hard flat implant	10
19	19/1	2000-19 A1/12	Hard flat implant	10
	19/3	2000-19 A2/12	Hard flat implant	15
	19/2	2000-19 B1/12	Hard flat implant	10
	19/4	2000-19 B2/12	Minimal lesion	5
20	20/1	2000-20 A1/12	Granulomatous cystic implant	10
	20/3	2000-20 A2/12	Flat lesion	10
	20/2	2000-20 B1/12	Cystic pyogranuloma	20
	20/4	2000-20 B2/12	Pyogranuloma	20

MACROSCOPICAL FINDINGS OF IMPLANTATION SITES

UPBRC Ref: H16/11 Our Ref: 2892/12

Date received: 19/03/2012

Macroscopical evaluation of implantation sites				
Rat no	UPBRC ref	Histo ref	Morphological Appearance	Size (mm)
1	1/1	2892-1 A1/12	Granuloma cystic	20
	1/3	2892-1 A2/12	Cystic	15
	1/2	2892-1 B1/12	Flat implant	20
	1/4	2892-1 B2/12	Granulomatous lesion	15
3	3/1	2892-3 A1/12	Flat implant	20
	3/3	2892-3 A2/12	Flat implant	20
	3/2	2892-3 B1/12	Minimal visible	10
	3/4	2892-3 B2/12	Minimal visible	10
4	4/1	2892-4 A1/12	Flat implant	15
	4/3	2892-4 A2/12	Flat implant	15
	4/2	2892-4 B1/12	Minimal visible	10
	4/4	2892-4 B2/12	Minimal visible	7
5	5/1	2892-5 A1/12	Flat implant	20
	5/3	2892-5 A2/12	Minimal lesion	5
	5/2	2892-5 B1/12	Minimal visible	3
	5/4	2892-5 B2/12	Minimal visible	10
6	6/1	2892-6 A1/12	Flat implant	15
	6/3	2892-6 A2/12	Flat implant	15
	6/2	2892-6 B1/12	Minimal visible	3
	6/4	2892-6 B2/12	Focal granuloma	10
7	7/1	2892-7 A1/12	No lesion	-
	7/3	2892-7 A2/12	No lesion	-
	7/2	2892-7 B1/12	No lesion	-
	7/4	2892-7 B2/12	Granuloma	20
8	8/1	2892-8 A1/12	Minimal visible	2
	8/3	2892-8 A2/12	Minimal visible	3
	8/2	2892-8 B1/12	Minimal visible	2
	8/4	2892-8 B2/12	Minimal visible	2
9	9/1	2892-9 A1/12	Minimal visible	2
	9/3	2892-9 A2/12	No lesion	-
	9/2	2892-9 B1/12	Flat implant	15
	9/4	2892-9 B2/12	No lesion	-



<i>Rat no</i>	<i>UPBRC ref</i>	<i>Lesion</i>	<i>Lesion Appearance</i>	<i>Size (mm)</i>
10	10/1	2892-10 A1/12	Flat implant	12
	10/3	2892-10 A2/12	Flat implant	10
	10/2	2892-10 B1/12	Minimal lesion	5
	10/4	2892-10 B2/12	Flat implant	15
11	11/1	2892-11 A1/12	No lesion	-
	11/3	2892-11 A2/12	Minimal lesion	3
	11/2	2892-11 B1/12	No lesion	-
	11/4	2892-11 B2/12	Cystic lesion	15
12	12/1	2892-12 A1/12	No lesion	-
	12/3	2892-12 A2/12	Focal pyogranuloma	15
	12/2	2892-12 B1/12	No lesion	-
	12/4	2892-12 B2/12	Flat and 2 nd pyogranuloma	10 - 15
13	13/1	2892-13 A1/12	Minimal lesion	2
	13/3	2892-13 A2/12	No lesion	-
	13/2	2892-13 B1/12	Flat implant	2
	13/4	2892-13 B2/12	Flat implant	5

RESULTS:

A. MORPHOLOGICAL FINDINGS OF THE STANDARD ORGANS SELECTED FOR HISTOLOGICAL EVALUATION

GROUP RECEIVED 21 FEBRUARY 2012: OUR REF 2000/12

RAT 14: OUR REFERENCE 2000-14/12

MACROSCOPICAL ANATOMICAL PATHOLOGY FINDINGS

1. No lesions are observed during macroscopical evaluation.
2. The animal is in good condition with normal body fat depots present.

HISTOPATHOLOGY

Adrenal:	Normal
Brain:	Sections from the brain which include both the cerebellum and the cerebrum do not show any neuropathology.
Gonad:	The ovary and female internal genitalia are normal.
Heart:	No lesions are observed in the myocardium.
Intestine, large:	No pathology is present.
Intestine, small:	Normal morphological features
Kidney:	No lesions are recorded.
Liver:	Normal morphological features. Mild congestion is visible.
Lung:	Mild congestion is observed. The peribronchial lymphoid tissue appears normal.
Pancreas:	No pathology is present.
Spleen:	The lymphoid tissue in the splenic white pulp appears normal while mild extramedullary haemopoiesis is observed in the red pulp. The red pulp is moderately congested.
Stomach:	No specific morphological changes are detected.
Thymus:	The thymic lymphoid tissue appears morphologically normal.
Urinary bladder:	Normal

COMMENT:

The examination is negative for specific pathology in the organs of this rat.

RATC 15: OUR REFERENCE 2000/15-12

MACROSCOPICAL ANATOMICAL PATHOLOGY FINDINGS

1. No lesions are observed in this animal.
2. The animal is in good condition with normal fat reserves visible.

HISTOPATHOLOGY

Adrenal:	No lesions are present in the adrenal sections.
Brain:	The histological evaluation does not show any lesions in the brain section. Mild leptomenigeal congestion is visible.
Heart:	No pathology is observed in the section from the heart muscle.



Gonad:	The ovary
Intestine, large:	No pathology is observed in the section from the large intestine. The gut-associated lymphoid tissue appears active.
Intestine, small:	No specific lesions are found in the small intestine.
Kidney:	No specific pathological changes are observed in the kidney section.
Liver:	No morphological pathology is visible.
Lung:	No abnormalities are present.
Pancreas:	Sections of the pancreas are morphologically normal.
Spleen:	Moderate haemosiderosis and extramedullary haemopoiesis are found.
Stomach:	Normal morphological features are present in the stomach section.
Thymus:	The thymic lymphoid tissue appears normal while no lesions could be confirmed in the interstitial stroma.
Urinary bladder:	No lesions are found.

COMMENT:

No specific morphological pathology is observed in the organs examined from this rat.

RAT 16 FEMALE: OUR REFERENCE 2000-16F/12

MACROSCOPICAL ANATOMICAL PATHOLOGY FINDINGS

1. No macroscopical lesions are found.
2. The stomach contains normal food contents.
3. Good body condition and normal fat depots are found.

HISTOPATHOLOGY

Adrenal:	The medulla is mildly congested.
Brain:	Normal
Gonad:	No pathology is observed in the section from the female internal genitalia.
Heart:	Normal morphology. No lesions are recorded.
Intestine, large:	No abnormalities are observed. Active gut-associated lymphoid tissue is normal.
Intestine, small:	No pathology is visible.
Kidney:	Mild medullary congestion could be demonstrated in the kidney section.
Liver:	No anatomical pathological changes present
Lung:	The section from the lung could not demonstrate any pathological changes in the pulmonary tissues.
Pancreas:	Normal architecture of the exocrine pancreas is present.
Spleen:	Normal splenic red pulp and white pulp are visible. Mild haemosiderosis is found.
Stomach:	No pathology is observed.
Thymus:	Mild congestion of the thymic medulla is present.
Urinary bladder:	Normal

COMMENT:

No specific lesions are detected in the organs examined from this rat.

**MACROSCOPICAL ANATOMICAL PATHOLOGY FINDINGS**

1. No specific pathology is observed in the organs examined.
2. The rat is in good body condition with normal fat depots visible.
3. Normal male genitalia observed.

HISTOPATHOLOGY

Adrenal:	Normal cortex and medulla are present.
Brain:	No lesions could be recorded in the several sections from the brain.
Gonad:	The testis sections appear normal and no lesions are observed in these sections. Normal spermatogenesis is confirmed.
Heart:	The myocardium appears morphologically normal and no lesions are present in the section from the heart.
Intestine, large:	The large intestine is normal without lesions visible in the mucosa and wall of the intestinal tract.
Intestine, small:	No pathology is observed.
Kidney:	The kidney shows only mild congestion.
Liver:	No lesions are observed.
Lung:	Mild focal atelectasis
Pancreas:	Normal
Spleen:	Active lymphoid tissue is present in the splenic white pulp while mild extramedullary haemopoiesis and haemosiderosis of the red pulp are found.
Stomach:	No lesions are recorded.
Thymus:	The thymic lymphoid tissue appears normal.
Urinary bladder:	No pathology is found in the urinary bladder.

COMMENT:

No specific lesions are observed. No lesions suggestive of intoxication are found.

RAT 17: OUR REFERENCE 2000-17/12**MACROSCOPICAL ANATOMICAL PATHOLOGY FINDINGS**

1. Mild congestion of the intestinal tract
2. The rat is in good body condition.

HISTOPATHOLOGY

Adrenal:	Normal cortex and medulla.
Brain:	Normal
Gonad:	Normal functional ovary and uterine horns
Heart:	No pathology is visible.
Intestine, large:	No lesions are observed.
Intestine, small:	Normal morphological features are found with mild congestion of the mucosa is present.
Kidney:	No pathological changes are detected.
Liver:	Normal histology
Lung:	No lesions are observed in the lung sections. The peribronchial lymphoid tissue appears active and normal.
Pancreas:	Normal pancreatic acini and ductal system are visible.
Spleen:	Normal splenic white pulp and red pulp with mild extramedullary haemopoiesis are detected.
Stomach:	No lesions are observed in the gastric mucosa and stomach wall.

Thymus:

The lymph node and medulla appears normal.

Urinary bladder:

No specimen



COMMENT:

No specific morphological anatomical pathology is present. Negative for any pathology in the organs examined.

RAT 18: OUR REFERENCE 2000-18/12

MACROSCOPICAL ANATOMICAL PATHOLOGY FINDINGS

1. No macroscopical pathological lesions are observed in the organs of this rat.
2. The female rat is in good body condition with fat reserves visible.

HISTOPATHOLOGY

Adrenal:	Normal morphological features are found.
Brain:	Normal
Gonad:	Normal inactive female genital organs.
Heart:	Negative for pathological lesions
Intestine, large:	No pathology is present.
Intestine, small:	Normal morphological features are visible.
Kidney:	No pathology is detected. Moderate congestion of the renal cortex is present.
Liver:	No lesions are found in the liver sections.
Lung:	Focal atelectasis is present.
Pancreas:	No lesions are observed.
Spleen:	Normal active splenic white pulp and normal red pulp are found.
Stomach:	No pathological changes are detected in the stomach wall including the mucosa
Thymus:	Mild congestion is detected.
Urinary bladder:	Normal morphology

COMMENT:

Specific pathological lesions are not observed in the organs examined from this rat

RAT 19: OUR REFERENCE 2000-19/12

MACROSCOPICAL ANATOMICAL PATHOLOGY FINDINGS:

1. The rat is in good body condition.

HISTOPATHOLOGY

Adrenal:	Normal
Brain:	Several sections were produced from the brain and show no morphological pathology.
Gonad:	Active productive ovary and normal internal tubular genitalia.
Heart:	No lesions are observed in the myocardium
Intestine, large:	Normal morphological features are present in the large intestine.
Intestine, small:	No morphological pathology is found.
Kidney:	Normal morphology
Liver:	No lesions are observed.
Lung:	No specific pathology is detected. Mild atelectasis is found.



Pancreas: No lesions
Spleen: Active white pulp is visible in the spleen section.
Stomach: The stomach appears morphologically normal
Thymus: The thymic lymphoid tissue is normal and no lesions could be observed in the interstitial stroma.
Urinary bladder: No lesions are recorded.

COMMENT:

The organs examined appeared normal.

RAT 20: OUR REFERENCE 2000-20/12

MACROSCOPICAL ANATOMICAL PATHOLOGY FINDINGS

1. The rat is in good body condition with fat depots visible.

HISTOPATHOLOGY

Adrenal: Congested
Brain: Normal
Gonad: The section from the ovary and uterus reveals no lesions.
Heart: No morphological pathological lesions are present.
Intestine, large: Normal morphology
Intestine, small: No lesions are observed.
Kidney: No pathology is visible.
Liver: The hepatic parenchyma appears morphologically normal.
Lung: Normal morphology is found.
Pancreas: Normal pancreas
Spleen: Moderate extramedullary haemopoiesis is observed in the splenic red pulp.
The splenic white pulp appears active and normal.
Stomach: No lesions are detected.
Thymus: The thymic lymphoid tissue and interstitial stroma are normal.
Urinary bladder No pathology is observed in the urinary bladder.

COMMENT:

Histological evaluation could not demonstrate any specific morphological pathology.



RAT 1: OUR REF 2892-1/12

HISTOPATHOLOGY

Adrenal gland:	Normal
Brain:	No lesions could be recorded in the neuroparenchyma and leptomeninges of the cerebral and cerebellar sections.
Gonad:	Numerous follicles and corpora lutea are present in the functional ovary. The uterus is normal.
Heart:	No lesions are observed in the myocardium.
Intestine, large:	Normal large intestinal morphology is visible in the different sections.
Intestine, small:	Normal morphological features
Kidney:	No lesions are found in the kidney section.
Liver:	No morphological microscopical pathology could be confirmed in the liver section.
Lung:	Mild atelectasis and emphysema are visible. Mild congestion is observed.
Pancreas:	Normal
Spleen:	The lymphoid tissue in the splenic white pulp appears normal while mild extramedullary haemopoiesis is observed in the red pulp. The red pulp is congested.
Stomach:	No specific morphological changes are detected.
Thymus:	Normal lymphoid tissue is present in the section.
Urinary bladder:	Normal mucosa and bladder wall

COMMENT

No specific anatomical pathological lesions are observed in the sections examined of this rat.

RAT 3: OUR REF 2892-3/12

HISTOPATHOLOGY

Adrenal gland:	Normal adrenal cortex and medulla
Brain:	No pathology could be confirmed in the sections from the cerebrum and cerebellum.
Gonad:	Normal functional ovary and uterus
Heart:	No pathology is observed in the section from the heart muscle.
Intestine, large:	Normal large intestinal mucosa is observed.
Intestine, small:	No specific lesions are observed in the small intestine.
Kidney:	Sections from both kidneys were evaluated without any tubular, interstitial and glomerular pathology present.
Liver:	No pathological changes could be observed in the hepatic parenchyma as well as the stromal tissue, portal tracts and biliary system of the liver.
Lung:	No abnormalities are observed. Mild atelectasis and active peribronchial lymphoid tissue are visible.
Pancreas:	The pancreatic acini, interstitial stromal tissue and islands of Langerhans appear morphologically normal.
Spleen:	Moderate congestion of the red pulp is found.
Stomach:	Normal morphological features are present in the stomach section.
Thymus:	Normal
Urinary bladder:	Normal

COMMENT

No specific morphological pathology could be demonstrated in this female rat.

RAT 4: OUR REF 2892-4/12

HISTOPATHOLOGY

Adrenal gland:	Normal
Brain:	Normal
Gonad:	The ovary and uterus of this female rat appears functional and normal.
Heart:	Normal morphology. No lesions are recorded.
Intestine, large:	No lesions could be confirmed in the large intestinal tract.
Intestine, small:	No pathology is visible.
Kidney:	Mild renal cortico-medullary congestion.
Liver:	No lesions are recorded in the sections from the liver.
Lung:	Focal mild atelectasis is found.
Pancreas:	Normal
Spleen:	Moderate congestion. Normal splenic red pulp and white pulp are visible
Stomach:	No pathology is observed.
Thymus:	No lesions are observed in the thymic lymphoid tissue and stroma.
Urinary bladder:	Normal

COMMENT

No diagnostic specific morphological pathology is observed during histopathology of the organs mentioned.

RAT 5: OUR REF 2892-5/12

HISTOPATHOLOGY

Adrenal gland:	Normal adrenal gland
Brain:	No neuropathology is observed in the neuroparenchyma with leptomeninges present.
Gonad:	Normal functional female internal genitalia
Heart:	No lesions are observed in the myocardium.
Intestine, large:	No pathology is observed in the large intestinal mucosa, gut-associated lymphoid tissue and intestinal wall
Intestine, small:	No pathology is present.
Kidney:	Sections were produced from the kidney and show only mild congestion.
Liver:	There is mild congestion and focal sinusoidal dilatation observed, but no lesions could be recorded in the hepatic parenchyma.
Lung:	Normal morphology of the lung.
Pancreas:	No pathology could be observed in the pancreatic sections.
Spleen:	Normal lymphoid tissue of the splenic white pulp is found with moderate congestion of the red pulp.
Stomach:	No lesions are recorded.
Thymus:	The thymic lymphoid tissue is morphologically normal.
Urinary bladder:	Normal morphology

COMMENT

No specific morphological pathology could be confirmed in this rat.

RAT 6: OUR REF 2892-6/12

HISTOPATHOLOGY

Adrenal gland:	Normal adrenal glands are observed.
Brain:	Pathology could not be confirmed in several sections of the brain of this rat.
Gonad:	The ovary, Fallopian ducts and uterus are all normal.
Heart:	No lesions could be confirmed, but there is mild congestion visible in the myocardium.
Intestine, large:	No lesions are observed.
Intestine, small:	Normal morphological features are present.
Kidney:	No pathological changes are detected.
Liver:	No microscopical pathological lesions could be recorded in the liver.
Lung:	No lesions are observed in the lung sections.
Pancreas:	Normal morphology, mild autolytic changes are found.
Spleen:	Normal splenic white pulp, red pulp and severe congestion are detected.
Stomach:	No lesions are observed in the gastric mucosa and stomach wall.
Thymus:	The thymic section reveals normal lymphoid tissue and stroma.
Urinary bladder:	Normal histology of the urinary bladder.

COMMENT

The macroscopical pathology and histopathology could not confirm any specific anatomical pathological changes.

RAT 7: OUR REF 2892-7/12

HISTOPATHOLOGY

Adrenal gland:	The adrenal glands appear morphologically normal.
Brain:	Several sections were produced from different areas in the brain and could not demonstrate any anatomical pathological lesions.
Gonad:	Normal and active functional ovary and tubular genitalia are present.
Heart:	Negative for pathological lesions
Intestine, large:	Normal gut associated lymphoid tissue is detected in the intestinal mucosa. No pathology is present.
Intestine, small:	Normal morphological features are visible.
Kidney:	No pathology is detected.
Liver:	No lesions are observed in the liver parenchyma as well as in the portal tracts.
Lung:	No lesions are found in the sections from the lungs. The peribronchial lymphoid tissue appears morphologically normal.
Pancreas:	Normal anatomical features are observed in the pancreatic section. No lesions are found.
Spleen:	Normal splenic white pulp and red pulp are present.
Stomach:	No pathological changes are detected in the stomach.
Thymus:	Normal
Urinary bladder:	Normal

COMMENT

The microscopical evaluation as well as the macroscopical necropsy did not show any specific pathological changes.



RAT 8: OUR REF 2892-8/12

HISTOPATHOLOGY

Adrenal gland:	Normal
Brain:	The microscopical evaluation could not detect any pathological changes in the neuroparenchyma and leptomeninges.
Gonad:	Normal internal female genitalia
Heart:	No lesions are observed in the myocardium
Intestine, large:	Normal morphological features are present in the large intestine.
Intestine, small:	No morphological pathology is present.
Kidney:	No pathological lesions are visible in the kidney sections.
Liver:	The liver appears morphologically normal and no microscopical pathological lesions are found.
Lung:	The lung alveoli and bronchial tree as well as the interstitial tissue appear morphologically normal.
Pancreas:	Normal morphology is visible.
Spleen:	Active white pulp is visible in the splenic section.
Stomach:	The stomach appears morphologically normal.
Thymus:	The thymic lymphoid tissue appears normal and the cortex and medulla of the thymic lobules are well-populated with lymphocytic cells.
Urinary bladder:	Normal histological morphology

COMMENT

The anatomical pathological evaluations do not reveal pathological changes that can be associated with this experiment.

RAT 9: OUR REF 2892-9/12

HISTOPATHOLOGY

Adrenal gland:	Normal
Brain:	Normal morphological features are seen in sections from the cerebrum and cerebellum.
Gonad:	The section from the ovary shows a normal functional organ. The uterus appears normal also.
Heart:	No morphological pathological lesions are present.
Intestine, large:	Normal morphology
Intestine, small:	No lesions are observed.
Kidney:	Mild renal congestion is present.
Liver:	Mild congestion is observed, but the rest of the hepatic parenchyma appears morphologically normal.
Lung:	Normal morphology is present.
Pancreas:	There are no pathological lesions observed in the pancreatic section.
Spleen:	Moderate congestion is observed in the splenic red pulp
Stomach:	No lesions are found.
Thymus:	The thymus is morphologically normal.
Urinary bladder:	Normal

COMMENT

Macroscopical and histological evaluation could not demonstrate any specific morphological pathology.

RAT 10: OUR REF 2892-10/12

HISTOPATHOLOGY

Adrenal gland:	The sections from both adrenal glands do not show any pathological changes.
Brain:	Normal
Gonad:	Normal ovary, Fallopian ducts and uterus are confirmed.
Heart:	No lesions are recorded in the myocardium.
Intestine, large:	The sections from the large intestine show normal intestinal mucosa and no parasites or any lesions could be demonstrated in the large intestine.
Intestine, small:	No pathology is detected.
Kidney:	Normal morphology
Liver:	The liver appears morphologically normal.
Lung:	Normal lung alveoli and airways are visible.
Pancreas:	Normal
Spleen:	Normal splenic white and red pulp. Moderate congestion is visible. Mild haemosiderosis is also present.
Stomach:	No pathology is observed.
Thymus:	Normal morphological features
Urinary bladder:	No pathology is found.

COMMENT

No specific lesions are detected in the organs from this rat.

RAT 11: OUR REF 2892-11/12

HISTOPATHOLOGY

Adrenal gland:	Normal
Brain:	Histological evaluation of different sections from the brain could not demonstrate any pathological lesions.
Gonad:	Normal ovarian and uterine architecture is present.
Heart:	Normal anatomical features. Mild congestion is found.
Intestine, large:	No lesions are recorded in the intestinal wall and mucosa.
Intestine, small:	No pathology is detected in the small intestine.
Kidney:	No lesions are observed in the kidney section.
Liver:	The liver appears morphologically normal and no lesions could be recorded in the liver sections.
Lung:	Sections from the lung show no pathological changes. The bronchial associated lymphoid tissue is normal.
Pancreas:	Normal morphology
Spleen:	Normal splenic white pulp is visible. Mild congestion and haemosiderosis are observed in the splenic red pulp.
Stomach:	No lesions are found in the gastric wall.
Thymus:	The lymphoid tissue of the thymus appears normal.
Urinary bladder:	Normal morphology observed.

COMMENT

No specific lesions are observed in the organs of this rat.

RAT 12: OUR REF 2892-12/12

HISTOPATHOLOGY

Adrenal gland:	Sections from the adrenal glands do not show any pathology.
Brain:	No lesions are recorded in several sections from the brain.
Gonad:	Normal functional female genitalia is present.
Heart:	Normal morphological features
Intestine, large:	The sections from the large intestine do not reveal any pathology.
Intestine, small:	No lesions are observed in the small intestinal sections.
Kidney:	The kidney is normal.
Liver:	The hepatic parenchyma and stromal tissues as well as portal tracts appear morphologically normal.
Lung:	The lung shows appears normal. No inflammation is present in the lung.
Pancreas:	Normal
Spleen:	Mild extramedullary haemopoiesis and congestion are found in the splenic red pulp.
Stomach:	No lesions are observed.
Thymus:	Normal lymphoid tissue is present in the thymic follicles.
Urinary bladder:	No pathology is found.

COMMENT

Evaluation of this rat does not show specific pathological changes.

RAT 13: OUR REF 2892-13/12

HISTOPATHOLOGY

Adrenal gland:	The adrenal glands are morphologically normal.
Brain:	No lesions are found.
Gonad:	Normal functional internal genitalia
Heart:	The myocardium is normal.
Intestine, large:	No pathology is detected.
Intestine, small:	Normal microscopical findings
Kidney:	No lesions could be observed in the kidney sections. Mild congestion is visible.
Liver:	No histological pathological changes could be confirmed in the section from the liver.
Lung:	Normal morphology of the lung is found.
Pancreas:	No lesions are found in the pancreas sections.
Spleen:	Moderate extramedullary haemopoiesis is visible in the splenic red pulp
Stomach:	Normal anatomy
Thymus:	The thymic lymphoid tissue and stroma appear morphologically normal.
Urinary bladder:	Normal

COMMENT

The anatomical pathology could not demonstrate specific lesions associated with the experimental procedure.

RESULTS

B: EVALUATION OF IMPLANTATION SITES

IMPLANTATION SITES OF GROUP RECEIVED 21ST OF FEBRUARY 2012: OUR REF 2000/12

See Table 2A attached.

Implanted material

The microscopical examination of the implantation sites in the rats received on 21 February 2012 is recorded in Table 2A. There were two types of implanted material consisting of osteoid matrix type implant where an eosinophilic homogenous stroma with lacunae of the osteocytic cells could be detected, and these eosinophilic implants resemble osteoid matrix of bone.

The second type of implant appears to be long filamentous threads of homogenous eosinophilic material without any cells or structures internally present. The threads show a constant width. This is recorded as filamentous implant in the tables.

Foreign body reaction

The foreign body reaction is part of the inflammatory process consisting mainly of epitheloid macrophages as well as multinucleated giant cells responsible for phagocytosis and resorption of foreign material such as the implanted osteoid and filamentous material or any other foreign material which includes hair etc. Small microgranulomas are observed around the implanted proteinaceous material, typical for a foreign body inflammatory reaction.

In this study the foreign body inflammatory reaction appears to be more prominent where the osteoid matrix implant was used when compared to those sites where a filamentous implant is visible.

Granulomatous reaction

The granulomatous inflammatory reaction is intermingled and present in and around both the osteoid matrix as well as the filamentous implants. It consisted of macrophages as well as mononuclear cells and few polymorphonuclear leukocytes around the implanted material. There appears to be minimal inflammatory oedema present and the granulomatous inflammation is localized only around the different implants.

The granulomatous inflammation is most prominent and more severe in the implantation sites where the osteoid matrix implant was identified in the majority of the implantation sites. The granulomatous inflammatory process could be identified in all of the different implantation sites as recorded in Table 2A.

Osteolysis

Osteolysis refers to the fragmentation and resorption of the eosinophilic proteinaceous implanted material, both osteoid matrix as well as the filamentous implant conducted by mainly osteoclastic cells and some of these osteoclasts are also multinucleated, similar to the multinucleated macrophages of the foreign body reaction. Osteoclasts accumulate, especially on the surface of the implanted material and is responsible for resorption of the eosinophilic proteinaceous implanted material.

The osteolytic process varies from mild to severe in the osteoid matrix implants, while it is minimal (negative) where the filamentous implant was used in this study.

Osteogenesis:

Osteogenesis refers to the process of new bone deposition which in this case would be intramembranous bone formation where existing osteoid matrix and implants are re-populated with osteocytic cells in the osteoid lacunae and proteinaceous stroma, while osteoblasts accumulate on the external surface of the proteinaceous stromal tissue and produce osteoid on the external surface of the membranous / proteinaceous eosinophilic material of the implant.

Osteogenesis appears to be absent in the majority of these implants sites and could be graded only as mild in a few of the osteoid matrix implant sites.

Ulcerated skin

The areas of ulceration are small and microscopically confirmed at the implantation site on the surface of the skin showing superficial exudative crusts and some areas of full-thickness epithelial necrosis, while mild inflammation is present in the dermis underneath the ulcerated skin. External irritation (scratching) may have a part in the pathogenesis.

Superficial ulceration is recorded in Table 2A and is found in both the osteoid matrix implant sites as well as the filamentous implant sites.

IMPLANTATION SITES OF GROUP RECEIVED 13 MARCH 2012: OUR REF 2892/12

See Table 2B attached. The grading of the histological findings in the implantation sites on the group of rats received on the 13th of March 2012 is similar to that reported above.

Implanted material

Again the two types of implanted material which is reported as osteoid matrix implant and filamentous implant were found in the implantation sites.

The filamentous implant was in some of the sites easily disrupted during tissue sectioning and is not so tightly adhered to the subcutaneous stroma as what is found with the osteoid matrix implant.

Foreign body reaction

A foreign body reaction could be confirmed in all of the implantation sites.

The foreign body reaction varied from mild to severe and there is little difference between the foreign body inflammatory process recorded for the osteoid matrix implants compared to the filamentous implants.

Granulomatous reaction

The granulomatous inflammatory process consisting of macrophages and epitheloid cells and also some lymphocytic inflammatory infiltrates which point towards a chronic inflammatory reaction. The body recognized the foreign material, while in some of the implantation sites, especially where the filamentous material was used, pyogranulomatous inflammation could be detected with the presence of numerous heterophils also visible in the central part of the granuloma.

Osteolysis

The osteolytic process of resorption and breakdown of the osteoid matrix and filamentous implant could be confirmed in most of the implantation sites as indicated in Table 2B. Disruption and resorption of the filamentous implant could be confirmed also in the majority of implantation sites.

Osteogenesis

The osteogenic or regenerative process with new bone development and re-population of osteoid lacunae with osteocytes was graded as mild and could be detected only in one of the osteoid matrix implant sites.

Ulcerated skin

Superficial ulceration in the epidermis was found in only three of the implantation sites, all of the osteoid matrix implant areas, while dermal scarring due to the implantation was present underneath these ulcers as well as in some of the non-ulcerated sites. This latter dermatofibrosis and scarring is part of the normal healing process where the osteoid matrix or filamentous implant was implanted into the subcutis.

DISCUSSION:

The morphological findings are recorded under the results, while grading of the pathological changes at the implantation sites are given in the tables attached.

These findings must be correlated with the history and while it appears in the earlier group of rats with our reference 2000/12 that the filamentous implantation sites show less of a foreign body reaction as well granulomatous inflammation and osteolysis, there is no prominent difference between this inflammatory process and osteolysis in the group of rats received later with our reference 2892/12. In this latter group the osteolytic process and resorption of the different implants are already advanced and complete healing should develop in a short time.

Osteogenesis could be demonstrated in some of the implantation sites of in the osteoid matrix group characterized by re-population of the lacuni with osteocytic cells and superficial osteoid production on the surface of the spicules of osteoid matrix that was implanted in the subcutaneous tissues. None of the filamentous implantation sites reveal any signs of osteogenesis.

Yours faithfully



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2012/04/04

For attention: Ilse Janse van Rensburg

**STUDY H16/11: SUBCUTANEOUS BIOASSAY OF NAVAL OSTEOCONDUCTIVE
PROTEIN MICROSTRUCTURE FOR ALTERNATIVE AUTOGRAFT BONE
TRANSPLANT SCAFFOLD: IDEXX REF 2000/12 AND 2892/12**

PROFORMA INVOICE / FAKTUUR	
1. Histopathology/Histopatologie x 70 @ R203 each	R 14210.00
2. Post mortem x 8 @ R203 each	R 1624.00
3. Handling fee	R 152.00
4. Photography: ½ hour	R 559.00
TOTAL / TOTAAL	R 16393.00

TABLE 2A: HISTOPATHOLOGICAL FINDINGS ON IMPLANTATION SITES: STUDY H16/11 OUR REF: 2000/12

TREATMENT	CC	CL	CH	KL	KP	KC	KG	KP	KG	CH	CH	CH	CC	KP	KC	CC
IMPLANT SITE	14/1	14/3	14/2	14/4	15/1	15/3	15/2	15/4	16/1M	16/3M	16/2M	16/4M	16/1F	16/3F	16/2F	16/4F
IDEXX REF	14A1	14A2	14B1	14B2	15A1	15A2	15B1	15B2	16A1	16A2	16B1	16B2	16A1	16A2	16B1	16B2
Implanted material	0	0	0	<i>f</i>	<i>f</i>	<i>f</i>	<i>f</i>	<i>f</i>	<i>f</i>	0	0	0	0	<i>f</i>	<i>f</i>	0
Foreign body reaction	2+	1+	2+	-	-	-	-	1+	-	2+	2+	3+	2+	2+	-	3+
Granulomatous reaction	3+	2+	3+	1+	1+	1+	1+	1+	1+	3+	3+	3+	3+	3+	1+	3+
Osteolysis	3+	1+	3+	-	-	-	-	-	-	1+	2+	2+	2+	-	-	2+
Osteogenesis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ulcerated skin	-	-	1+	2+	1+	2+	1+	-	-	-	1+	1+	2+	1+	1+	1+

Legend:

0	Osteoid matrix implant (collagen)	CC	Collagen control	KG	Kafirin microparticle film treated with glutaraldehyde
<i>f</i>	Filamentous implant (kafirin microparticle film)	CL	Collagen low BMP-2 loading	KP	Kafirin microparticle film treated with polyphenol
-	Negative / No Lesion	CH	Collagen high BMP-2 loading		
1+	Mild	KC	Kafirin microparticle film control		
2+	Moderate	KL	Kafirin microparticle film low BMP-2 loading		
3+	Severe	KH	Kafirin microparticle film high BMP-2 loading		

TABLE 2B: HISTOPATHOLOGICAL FINDINGS ON IMPLANTATION SITES: STUDY H16/11 OUR REF: 2892/12

TREATMENT	CC	CC	CC	CC	CL	CL	CL	CL	CH	CH	CH	CH	KC	K C	KC	K C	KL	KL	KL	KL	K H	K H	KH	K H
IMPLANT SITE	4/2	6/2	5/3	12/ 1	13/1	5/4	8/1	4/4	11/3	13/2	7/3	1/4	1/3	6/ 4	10/ 1	9/ 3	12/ 3	7/ 1	3/ 1	11/ 4	3/ 4	8/ 4	9/2	10 /3
IDEXX REF	4B 1	6B1	5A2	12A 1	13A 1	5B2	8A1	4B2	11A 2	13B 1	7A2	1B 2	1A 2	6B 2	10 A1	9A 2	12 A2	7A 1	3A 1	11 B2	3B 2	8B 2	9B 1	10 A2
Implanted material	0	0	0	0	0	0	0	0	0	0	0	0	<i>f</i>	<i>f</i>	<i>f</i>	<i>f</i>	<i>f</i>	0	<i>f</i>	<i>f</i>	<i>f</i>	<i>f</i>	<i>f</i>	<i>f</i>
Foreign body reaction	3+	3+	3+	1+	2+	2+	2+	2+	2+	2+	3+	3+	2+	1+	2+	1+	2+	1+	2+	2+	2+	2+	2+	1+
Granulomatous reaction	3+	3+	3+	2+	3+	1+	2+	3+	2+	2+	1+	3+	2+	3+	2+	1+	3+	1+	3+	2+	2+	1+	3+	2+
Osteolysis	2+	2+	3+	2+	1+	2+	1+	2+	2+	2+	2+	3+	3+	2+	2+	-	1+	-	2+	2+	2+	-	1+	1+
Osteogenesis	-	-	-	-	-	-	-	-	-	-	-	1+	-	-	-	-	-	-	-	-	-	-	-	-
Ulcerated skin	-	1+	-	-	-	1+	-	-	1+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Legend:

0	Osteoid matrix implant (collagen)	CC	Collagen control
<i>f</i>	Filamentous implant (kafirin microparticle film)	CL	Collagen low BMP-2 loading
-	Negative / No Lesion	CH	Collagen high BMP-2 loading
1+	Mild	KC	Kafirin microparticle film control
2+	Moderate	KL	Kafirin microparticle film low BMP-2 loading
3+	Severe	KH	Kafirin microparticle film high BMP-2 loading

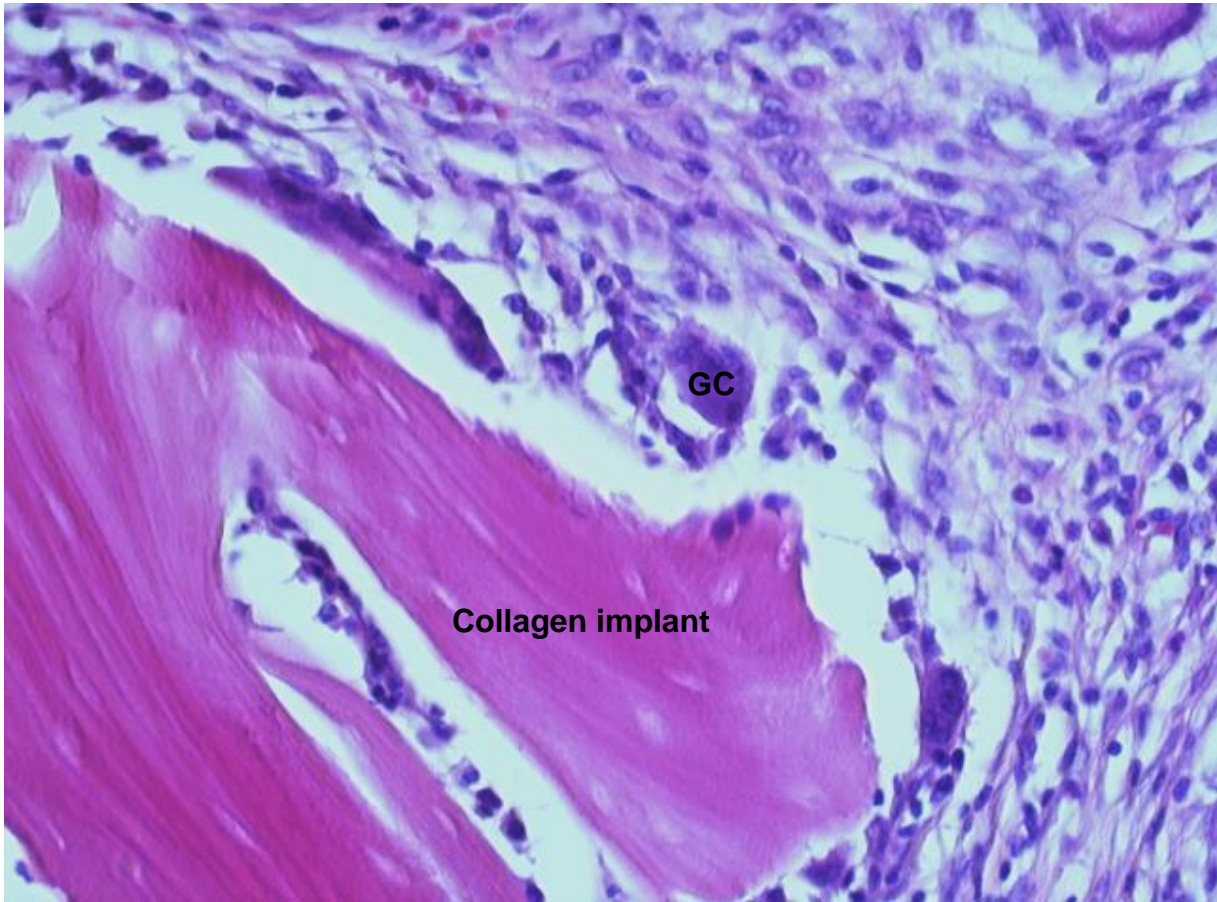


Figure A1. Image of haematoxylin–eosin stained sections of collagen implant in the subcutaneous site showing foreign body giant cells Day 7 post implantation. **GC**-Giant cell. Magnification x 200

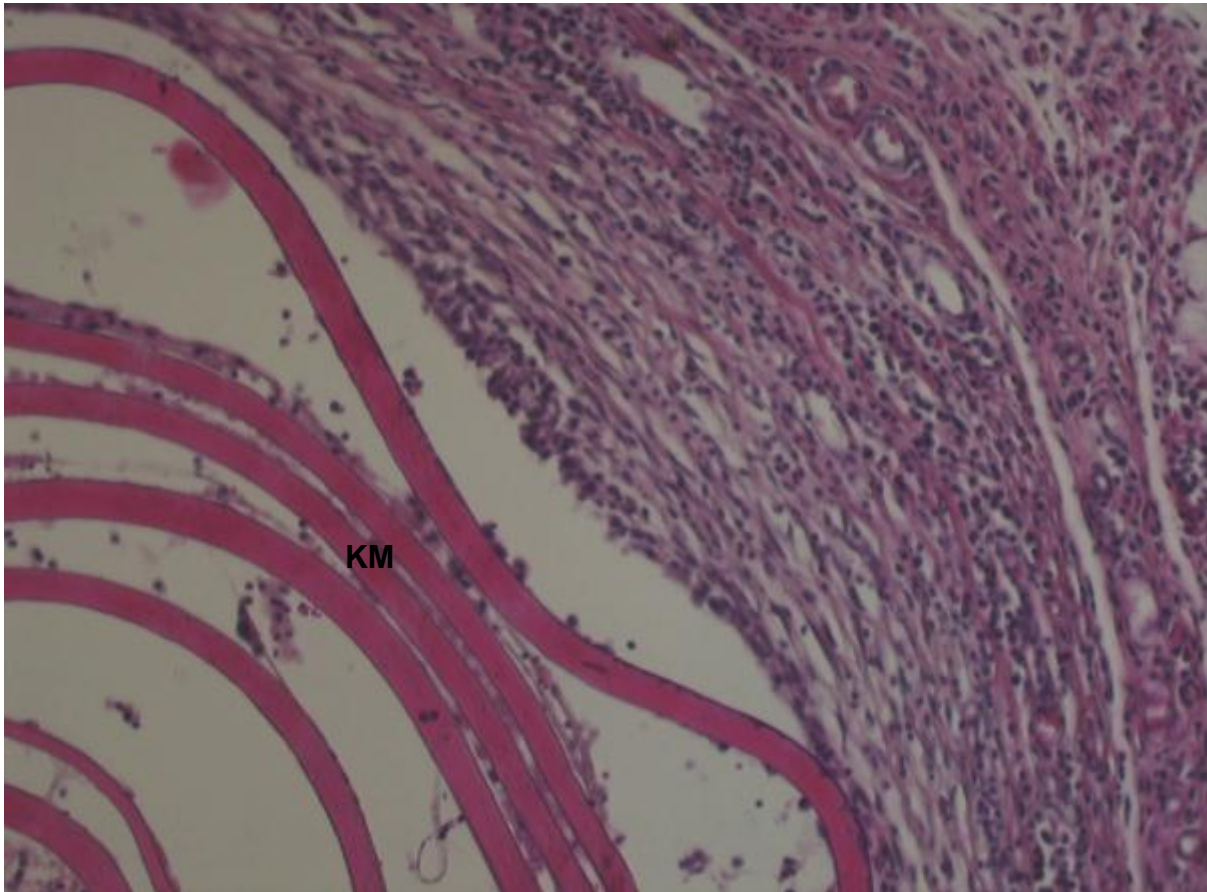


Figure A2. Image of haematoxylin–eosin stained sections of kafirin microparticle film implants in the subcutaneous site showing intact kafirin microparticle film implant and inflammation by Day 28 post implantation. **KM** - Kafirin microparticle film implant. Magnification x 100.

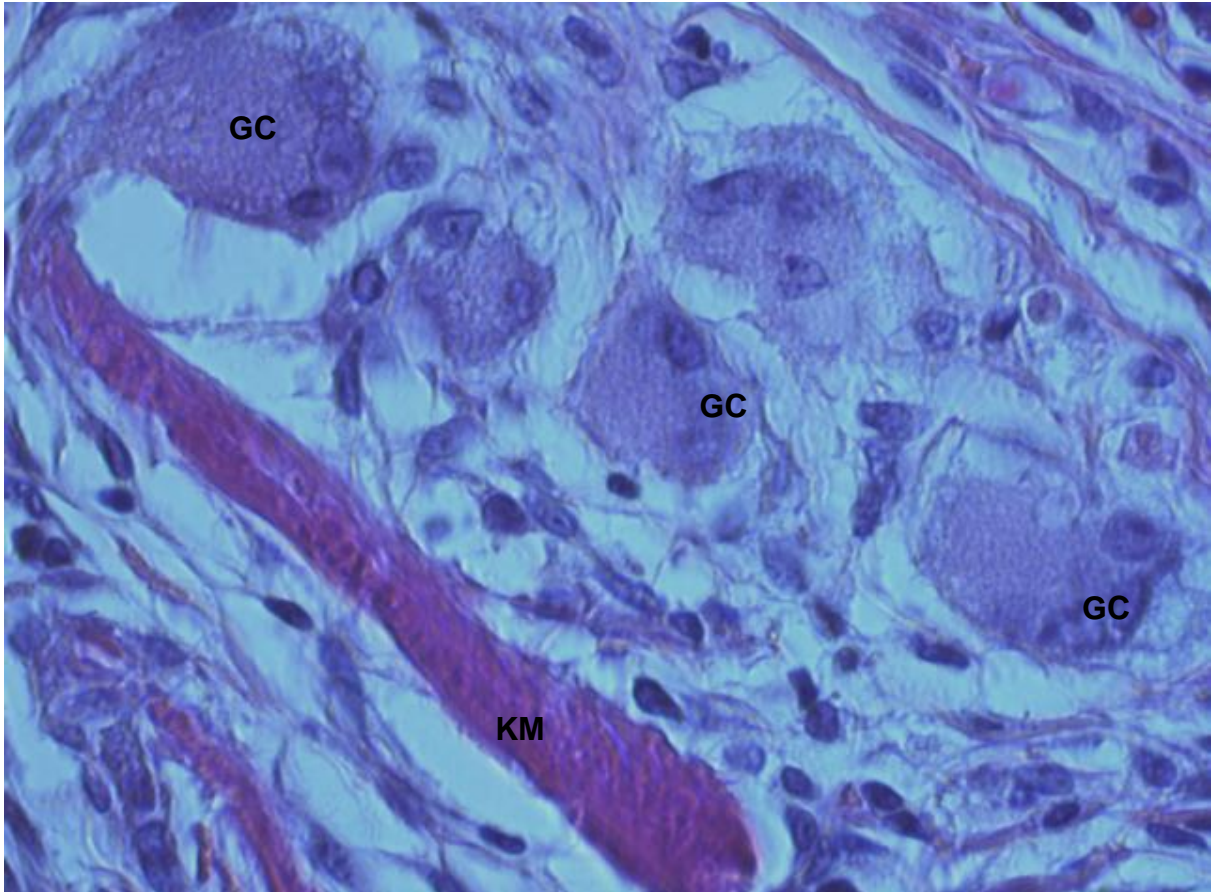


Figure A1. Image of haematoxylin–eosin stained sections of kafirin microparticle film implant in the subcutaneous site showing foreign body giant cells Day 28 post implantation. **KM-** kafirin microparticle film implant. **GC-**Giant cell. Magnification x 400.

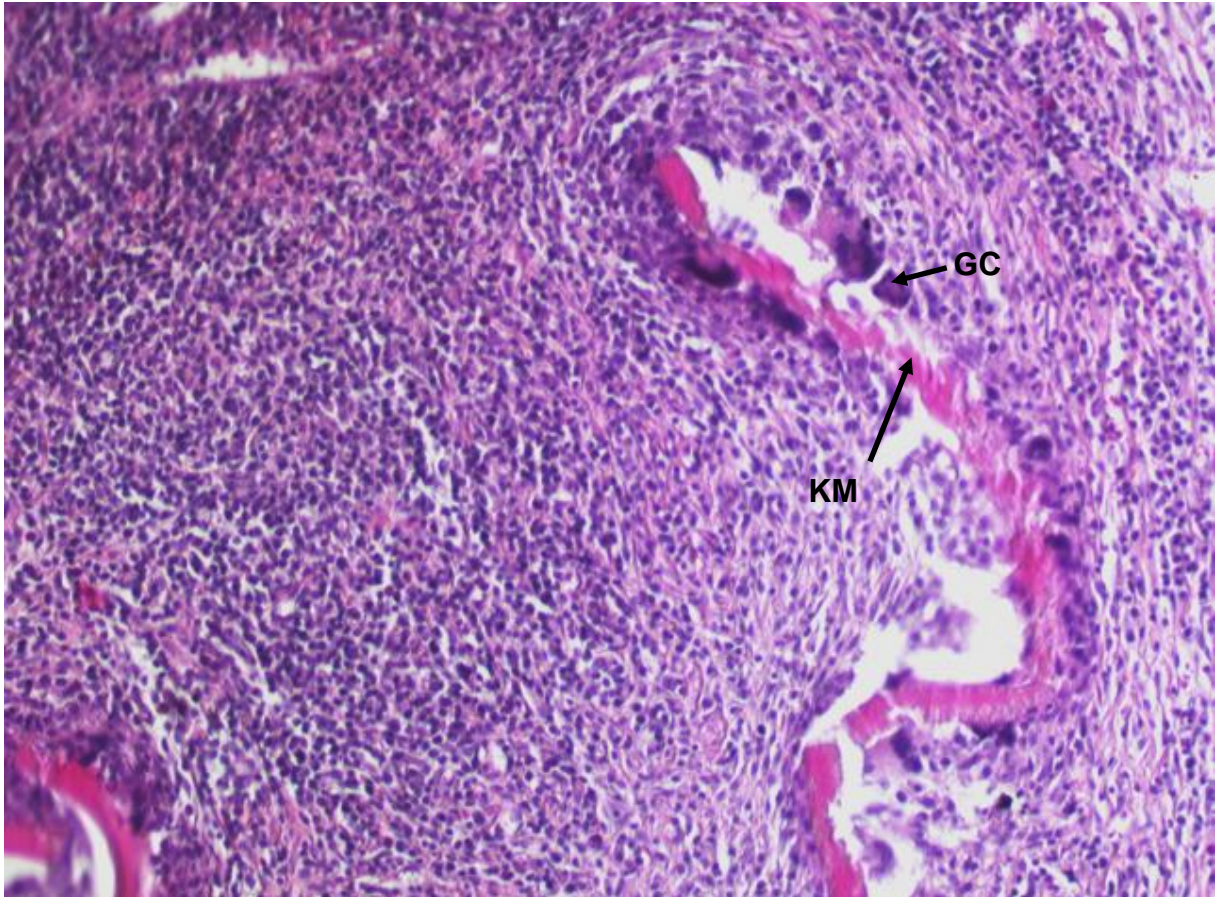


Figure A2. Image of haematoxylin–eosin stained sections of kafilin microparticle film implant in the subcutaneous site showing lysis (degradation) of kafilin microparticle film and inflammation by Day 28 post implantation. **KM** – Kafilin microparticle film implant **GC**- Giant cell. Magnification x 100.

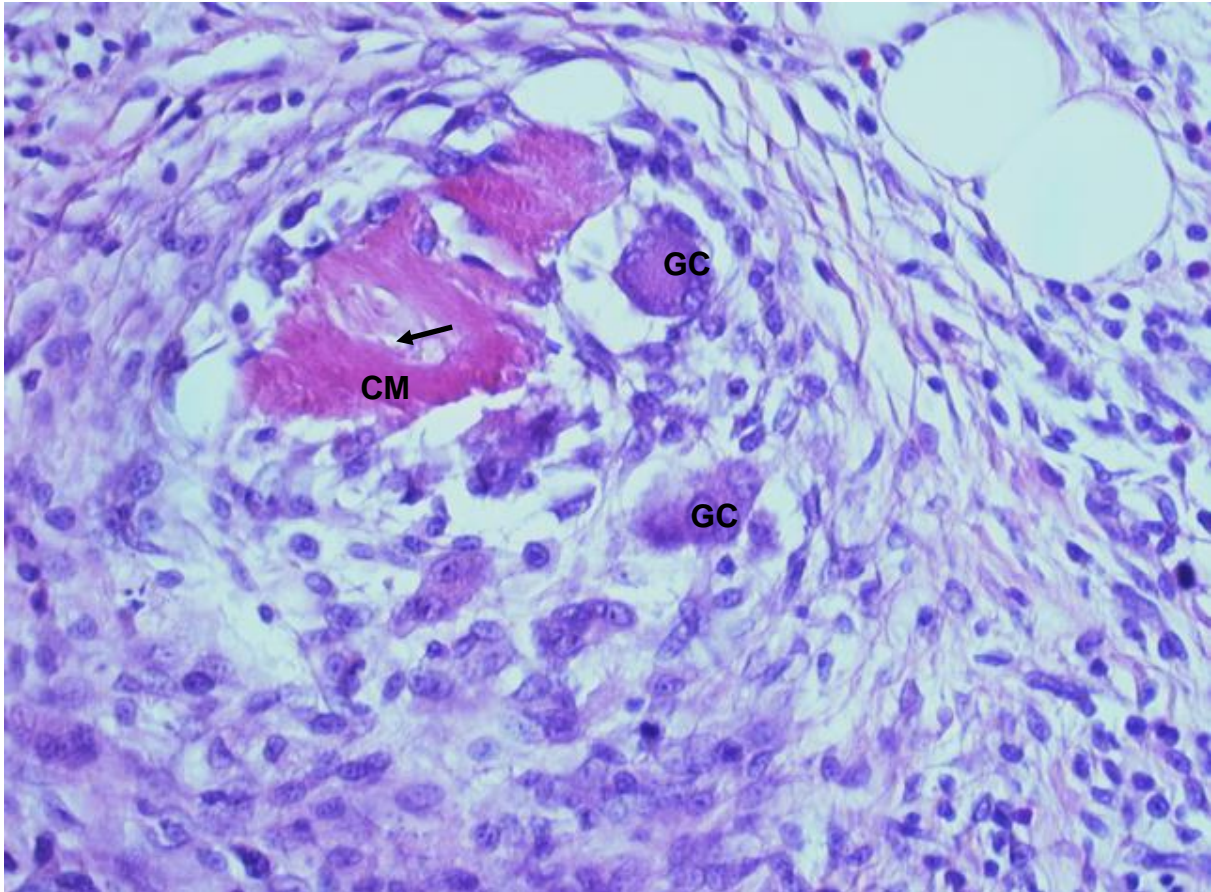


Figure A3. Image of haematoxylin–eosin stained sections of collagen implant in the subcutaneous site showing Osteolysis of collagen implant Day 7 post implantation. **CM**-Collagen implant. **GC**-Giant cell. Arrow is pointing at site of osteolysis. Magnification x 200.

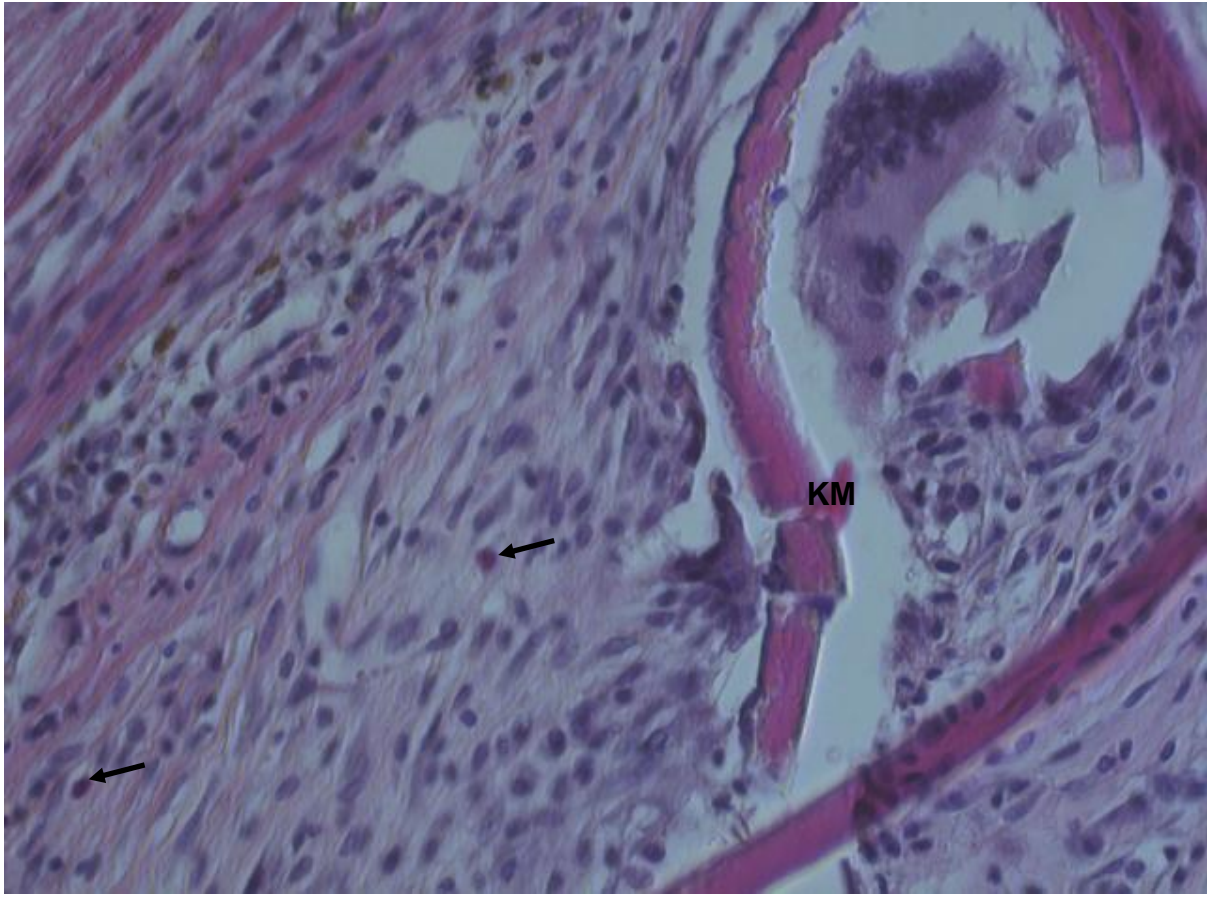


Figure A4. Image of haematoxylin–eosin stained sections of kafirin microparticle film implant in the subcutaneous site showing granulomatous inflammatory reaction with kafirin microparticle film implant 28 days post implantation. **KM** – kafirin microparticle film implant. Arrows pointing at heterophils, which indicate granulomatous inflammatory reaction. Magnification x 200.

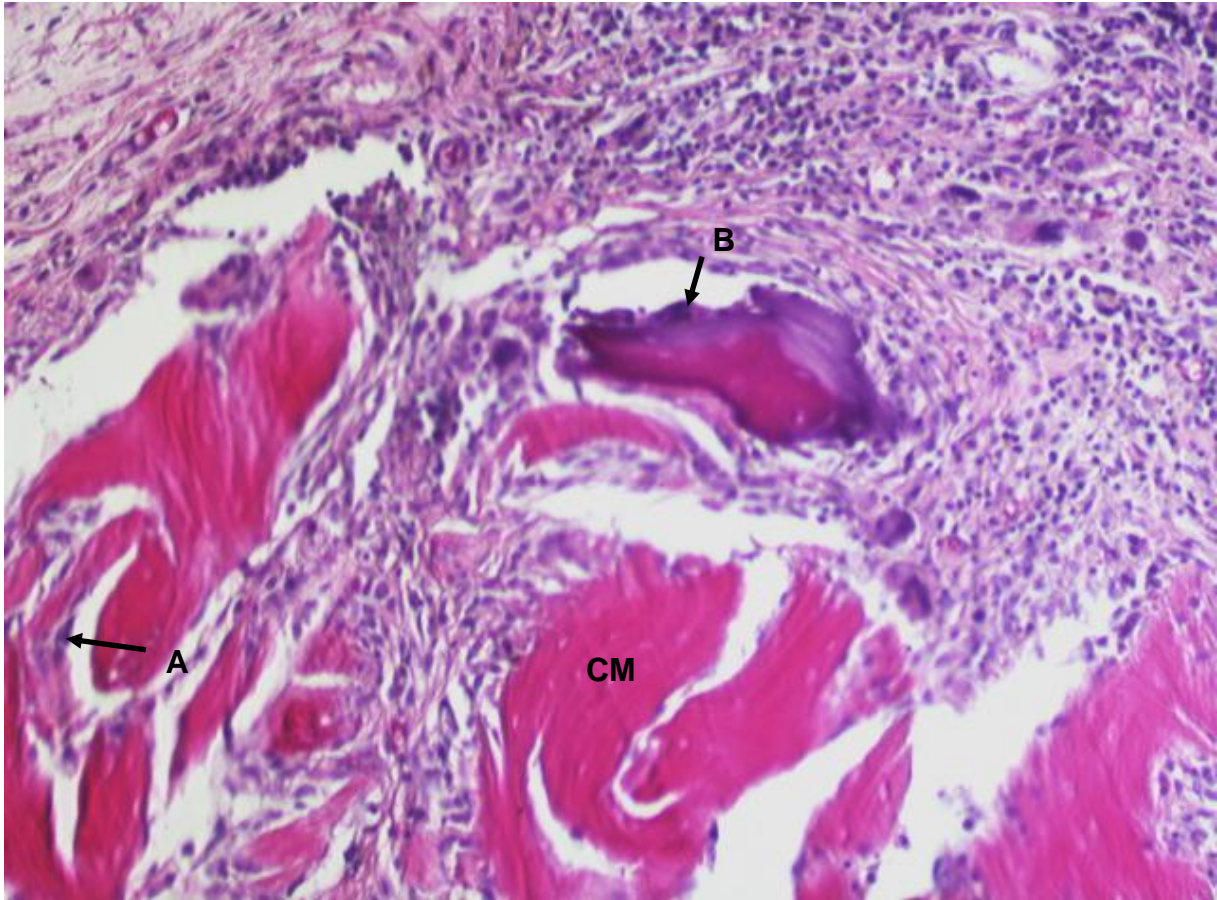


Figure A5. Image of haematoxylin–eosin stained sections of collagen implant showing mild osteogenesis after the implantation for 28 days. CM -Collagen implant. **A.** Shows the repopulation of the osteoid lacunae with osteocytes. **B.** Bluish staining at the edge of the collagen implant indicate presence of osteoblasts. Magnification x 100.