Jellagen

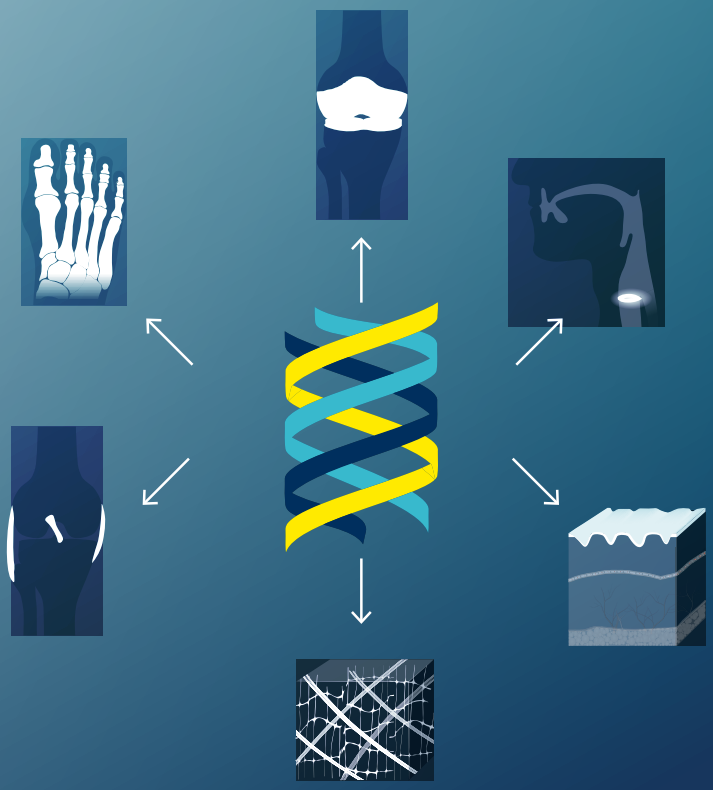
NEXT GENERATION COLLAGEN

Collagen Type 0

A revolutionary biomaterial, 600 million years in the making

Summary

- Jellyfish collagen (Collagen Type 0) is ancestrally ancient and far simpler than mammalian collagens
- It is derived from jellyfish species, including *Rhizostoma pulmo*
- Collagen Type 0 is biocompatible, and triggers tissue regeneration rather than inflammation due to its unique macrophage response
- Potential clinical applications include treatment of chronic wounds, osteoarthritis and vocal cord paralysis, as well as many more indications



COLLAGEN Type 0 By Jellagen

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Where does Collagen Type 0 come from?

It is a collagen derived from jellyfish species, such as *Rhizostoma pulmo* (Fig. 1), and is an ancestral form of this prevalent structural protein found in the connective tissue of organisms. Collagen from jellyfish predates the mammalian form by around 600 million years, when jellyfish first came into existence. Mammalian collagen is highly specialized to satisfy the biomechanical requirements of mammals' complex physiological systems. For example, Type 1 collagen is associated with skin and bone and Type 2 collagen is associated with cartilage tissues. Jellyfish collagen, on the other hand, has retained a greater degree of chemical simplicity leading to greater tissue multi-functionality and structural versatility. For this reason, jellyfish collagen has been categorized as Collagen Type 0 (sometimes also referred to as JFC_0).

R. pulmo, commonly known as the barrel jellyfish, is found in huge blooms in numerous oceans and seas across the globe. Jellagen Ltd harvest *R. pulmo* naturally off the west coast of Wales and manufacture the highly purified Collagen Type 0 products at their ISO 13485:2016 facility in Cardiff, UK. Collagen Type 0 is a sustainable biomaterial, with a low carbon footprint, given that *R. pulmo* is a naturally occurring pest species. The rapid expansion in *R. pulmo* numbers seen in recent decades, as well as other jellyfish species, is an unfortunate consequence of over-fishing, rising water temperatures and plastic pollution[1]. Collagen Type 0 has the potential to be manufactured from any jellyfish species at huge scale.

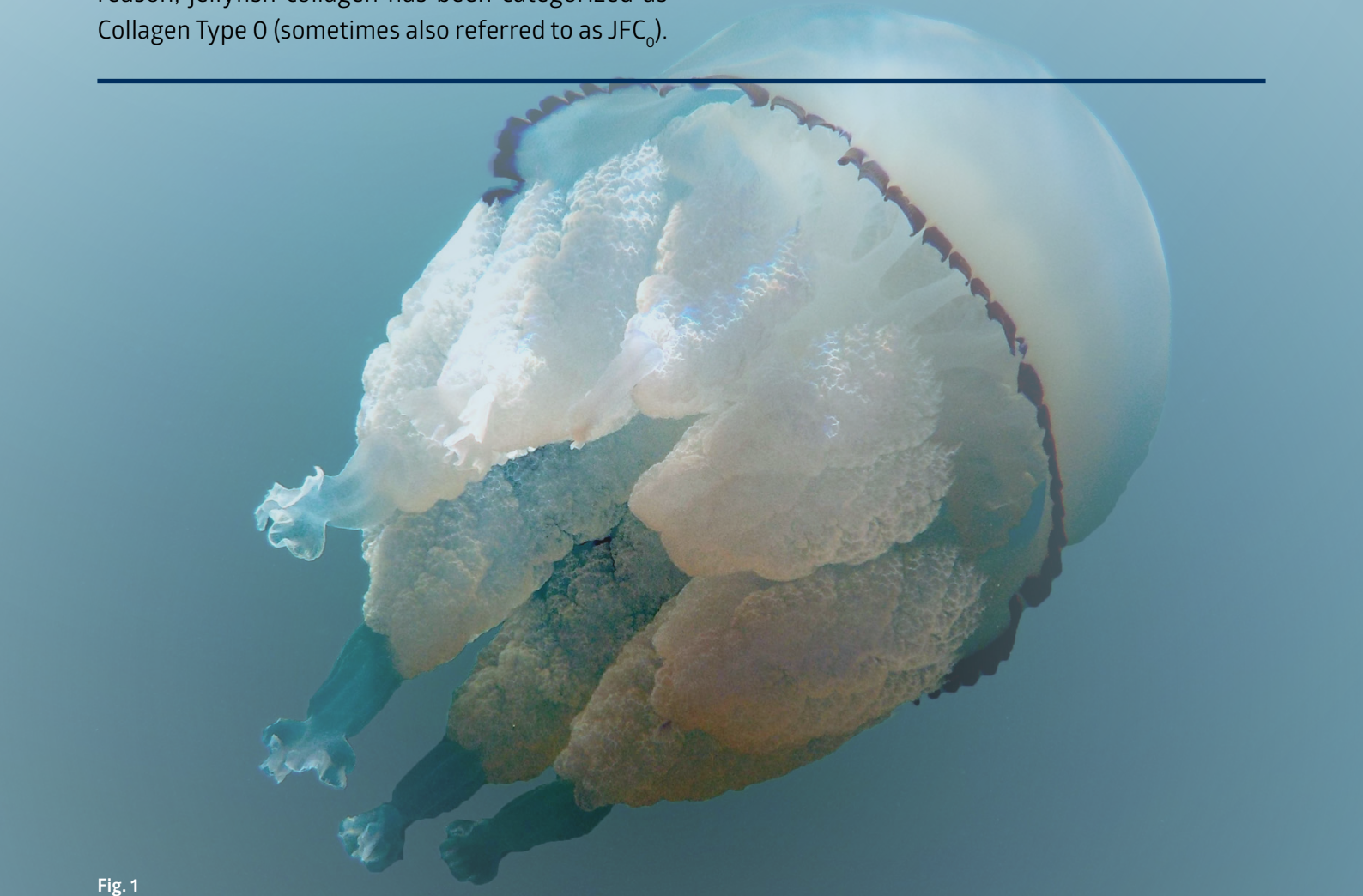


Fig. 1

***Rhizostoma pulmo* (barrel jellyfish)**

Properties of Collagen Type 0

Collagen Type 0 is a triple helical protein, as is typical with all collagen proteins. It is structurally versatile, i.e. it can be formulated into different structures such as porous scaffolds, hydrogels or other formulations.

Types of collagen

Twenty-eight different types of collagens have been identified in mammals. The most common types in humans are Types I, II, III, IV and V. These can be found in the following locations:

Type I:	skin, tendon, blood vessels, organs, bone, cornea
Type II	cartilage
Type III:	reticular fibers found in connective tissue of organs
Type IV:	basement membrane in various tissues
Type V:	cell surfaces, cornea, hair, placenta

Collagen Type 0 from jellyfish is similar to Types I, II, III and V.

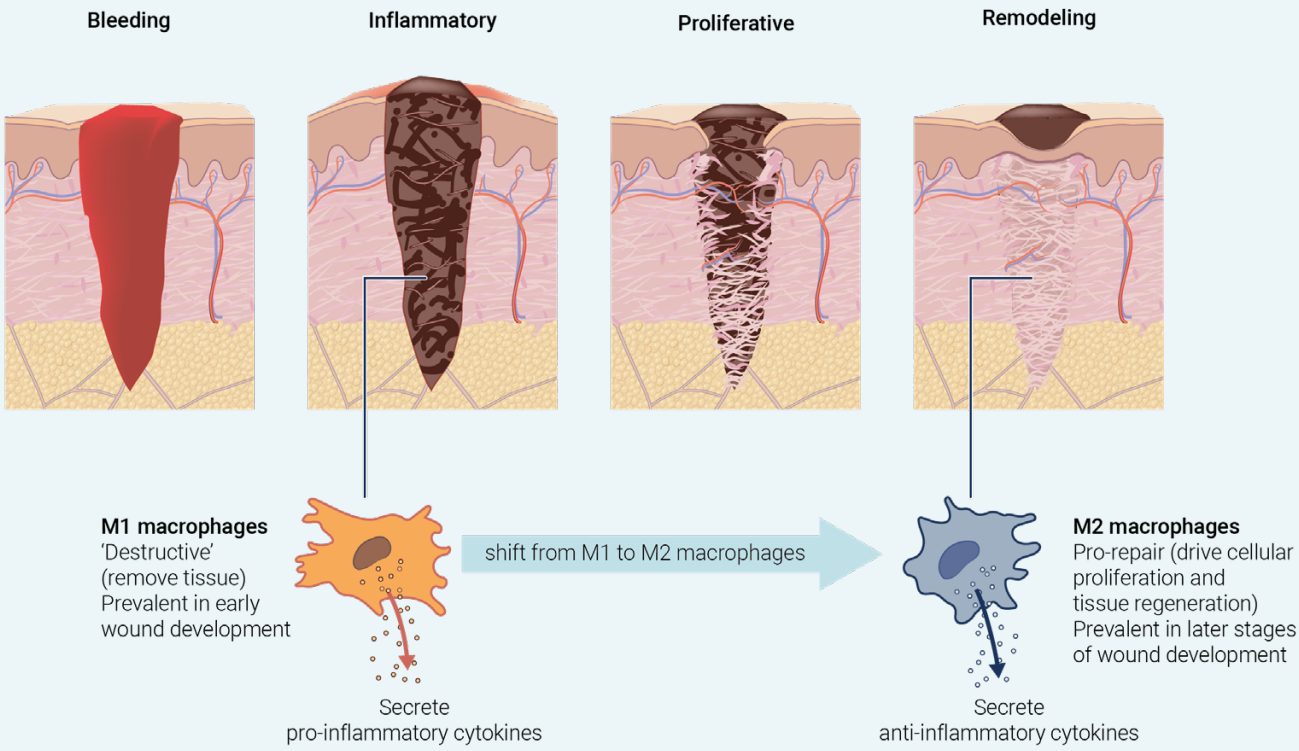
One of the most interesting properties of Collagen Type 0 is its ability to trigger regeneration in tissues, as opposed to inflammation. This can be explained at a cellular level by the balance between two different types of macrophages: M1 and M2 macrophages. Collagen Type 0 triggers a lower M1 macrophage response and a higher M2 macrophage response than other mammalian collagens. The positive tendency for less inflammation and more structured tissue regeneration with Collagen Type 0 has been seen in models for wound healing, bone regeneration and treatment of vocal cord paralysis.

Macrophages

A macrophage is a type of white blood cell of the immune system. They are involved in 'non-specific immunity'. This is where macrophages act as scavengers to engulf and destroy other cells, bacteria, and microscopic particles, in a process known as phagocytosis. They also play a role in 'specific immunity' to alert the immune system to invading microbes. Here, after ingesting an invading microbe, the macrophage 'presents' a protein from the microbe (an antigen) on its surface. The antigen is recognized by T cells (a type of lymphocyte), which activate other cells of the immune system, and initiate inflammation. This process is controlled by the release of cytokines from the macrophage.

Fig. 2

M1 and M2 macrophages in normal acute wound healing



In acute wounds, as depicted in the figure above, there is a shift from M1 to M2 macrophages over time (and a corresponding shift from inflammation and tissue removal, to tissue repair).

However, in chronic wounds, the development of mostly M1 macrophages results in dysregulated inflammation and poor wound healing and closure. This can lead to potential infections and more severe conditions.

Potential benefits of Collagen Type 0

Due to its ancient ancestry and simple nature, Collagen Type 0 is biocompatible in mammals. The unique macrophage response to Collagen Type 0, described previously, also means that it has potential to enhance healing and improve closure of wounds, with less scarring, as already seen at a histological level (Fig. 3). In turn, this can mean the prevention of infections in chronic wounds, as well as further complications.

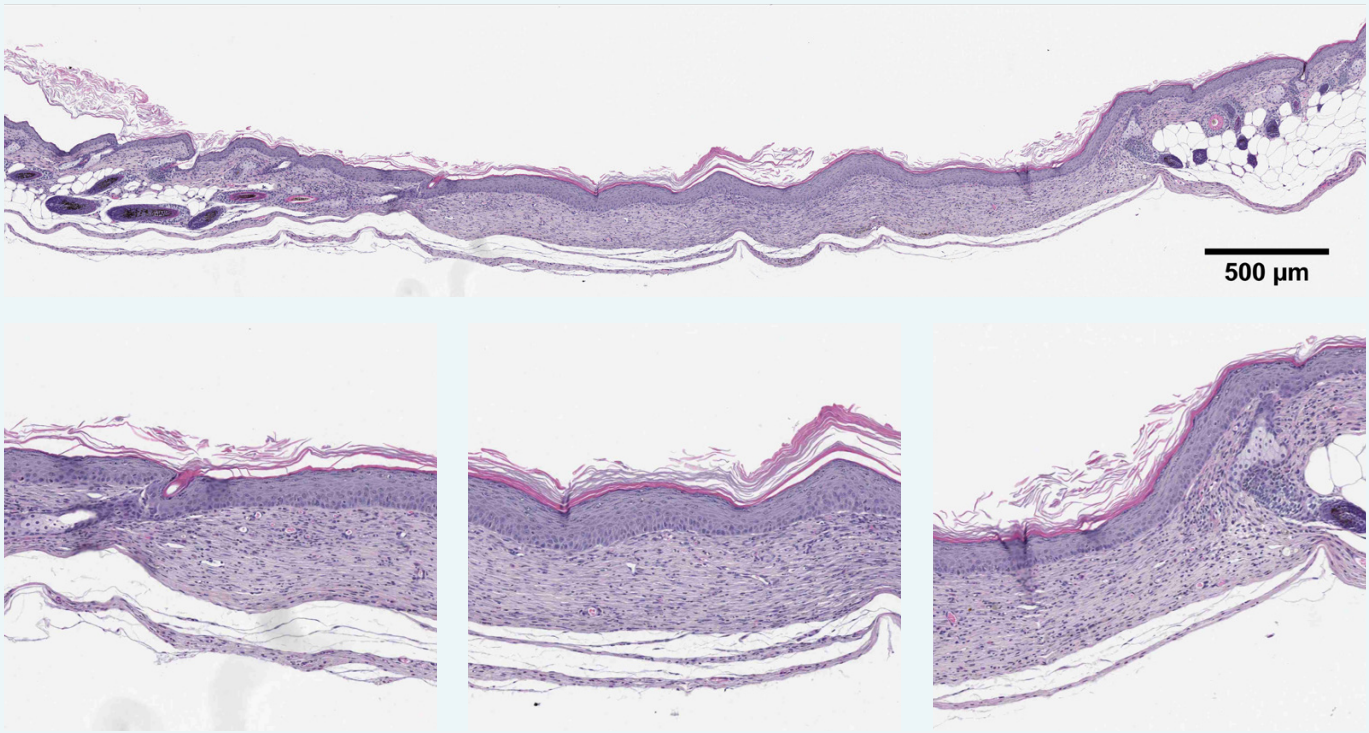


Fig. 3

Histology image

Histology studies have shown Collagen Type 0 leads to the formulation of organized tissue in wound healing, with little scarring.

What does the data show?

Study 1) Biocompatibility and anti-inflammatory properties of Collagen Type 0 (*in vitro*)

In this *in vitro* study the effect of jellyfish Collagen Type 0 on differentiated THP-1 cells, a type of cell associated with wound development, was evaluated[2]. A previous study reported that collagen extracted from other jellyfish species (*Nemopilema nomurai*) may stimulate the secretion of pro-inflammatory cytokines (TNF-α / IL-6)[3]. We therefore wanted to see if Collagen Type 0 from *R. pulmo* shows a similar or different effect.

What are THP-1 cells?
THP-1 cells are used as a model for monocytes, a type of white blood cell (leukocyte). Monocytes migrate to infection site in response to inflammation signals. They can then differentiate into macrophages or dendritic cells to affect an immune response.

Inflammatory cytokine secretion
The secretion of TNF-α, together with other cytokines associated with inflammation (IL-8, IL-1β and IL-18), were evaluated in the differentiated THP-1 **cells alone** or when **Collagen Type 0** was present. It was found that Collagen Type 0 did not induce significant changes in the secretion of pro-inflammatory cytokines (Fig. 4).

Furthermore, the differentiated THP-1 cells (both **alone** and when **Collagen Type 0** was present) were challenged with lipopolysaccharide (LPS). LPS is a major component of gram-negative bacteria and is known to trigger an inflammatory immune response. Introduction of LPS led to a marked inflammatory response, but the presence of Collagen Type 0 did not alter this is any way (Fig. 5).

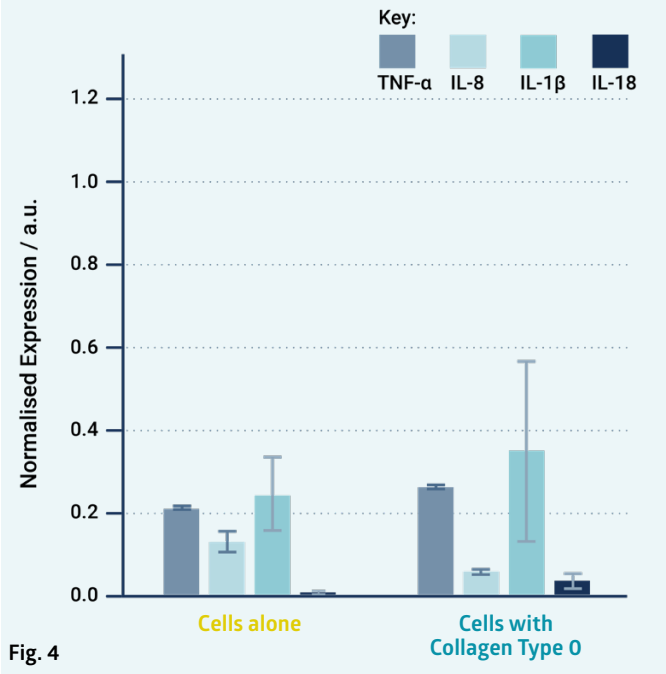


Fig. 4

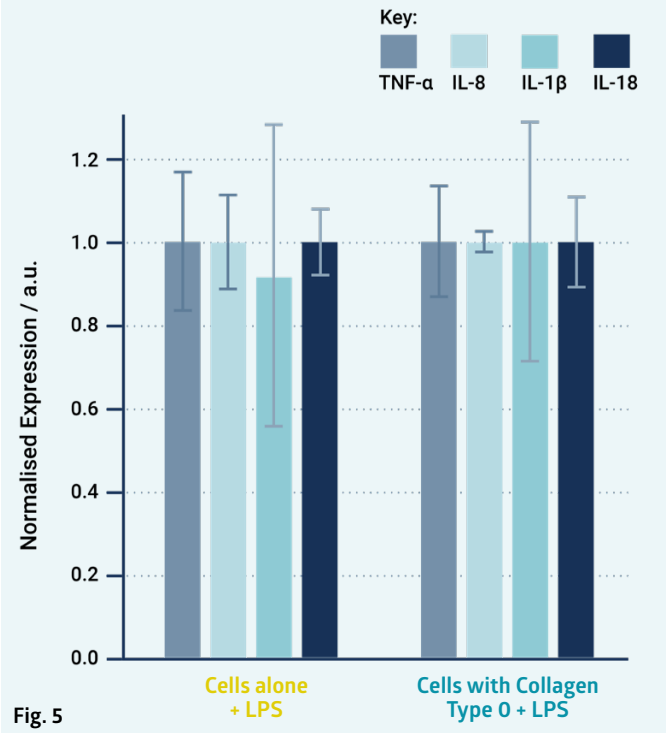


Fig. 5

Key takeaway: Collagen Type 0 shows excellent biocompatibility and anti-inflammatory properties.

Study 2a) Are the anti-inflammatory properties of Collagen Type 0 also seen *in vivo*?

A 2020 study by Flaig I. *et al.* examined the macrophage response to Collagen Type 0 implanted subcutaneously in Wistar rats[4]. This was compared with a control group of rats, which had porcine pericardium collagen (PPC, botiss biomaterials, GmbH, Zossen, Germany) implanted. Macrophage response (both total macrophage numbers and the balance between M1, or CD11c-positive, macrophages, and M2, or CD163-positive, macrophages) was assessed after 10 and 30 days.

Total macrophage numbers

There were significantly lower numbers of total macrophages in response to **Collagen Type 0** compared to **PPC** at both days 10 and 30 ($p<0.001$) (Fig. 6). Total macrophage numbers did not significantly decrease between day 10 and day 30 with **PPC**, while there was a significant two-fold reduction with **Collagen Type 0** ($p<0.05$)*.

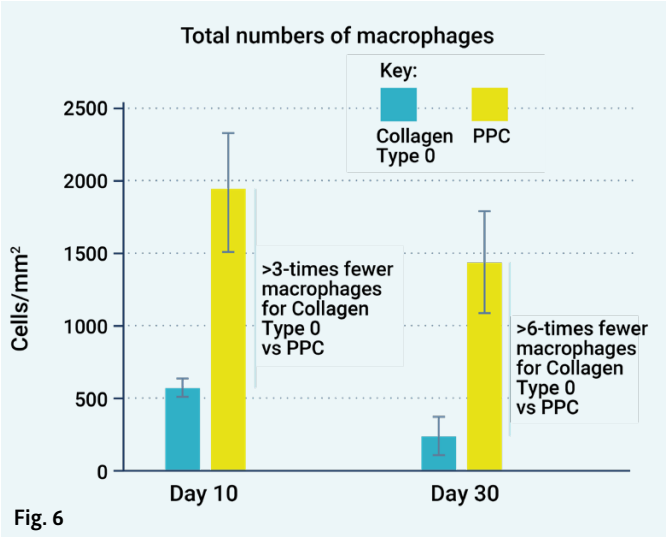


Fig. 6

M1 and M2 macrophage balance

Looking at the balance between M1 and M2 macrophages, there were significantly more M2 macrophages than M1 at day 10 with **Collagen Type 0** ($p<0.05$) (Fig. 7). By day 30, total macrophages had decreased and there was no significant difference between the two types present in the wound bed. With **PPC**, there was only a shift to predominantly M2 macrophages after 30 days*. Typically, in a wound, most healing occurs by day 30 and the body will go into an equilibrium state.

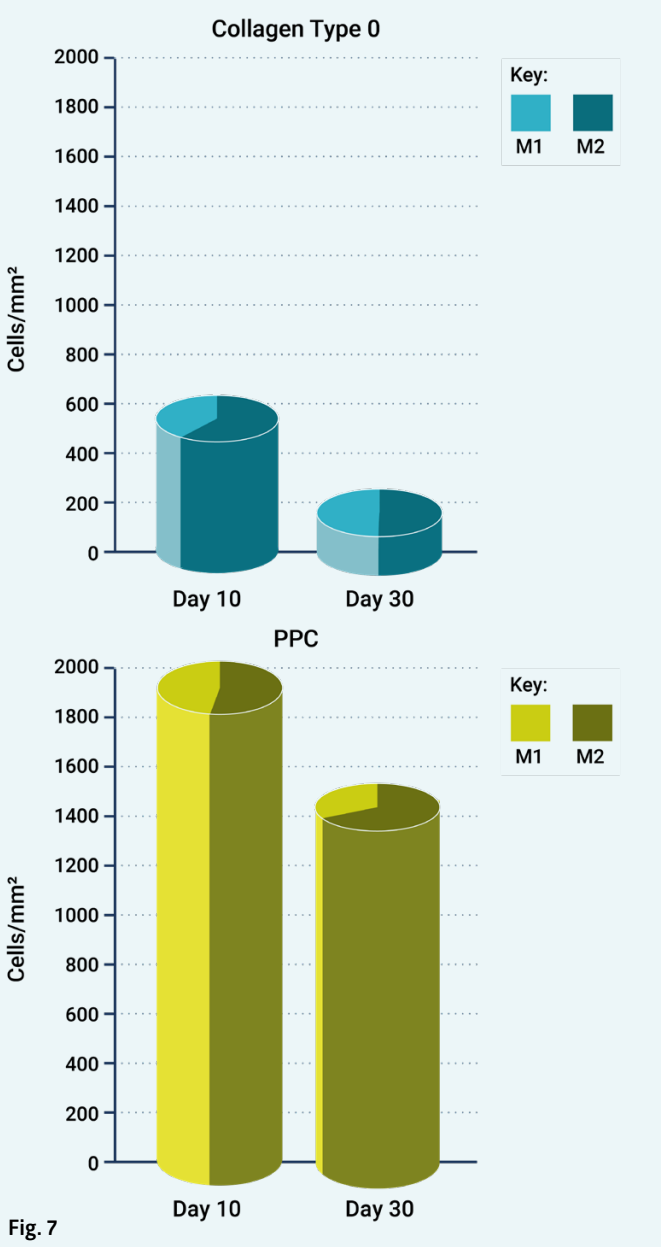


Fig. 7

Key takeaway: Collagen Type 0 shows a weaker immune tissue response than PPC; Collagen Type 0 is therefore biocompatible. The macrophage balance shows that Collagen Type 0 triggers tissue repair (as characterized by M2 macrophages) rather than inflammation (as characterized by M1 macrophages) early in wound healing. Tissue repair is only stimulated late in wound healing with PPC.

*Results from an unpublished sub-analysis of the data

Study 2b) Impact on healing of a cranial wound

The second part of the Flaig I. *et al.* study examined whether the anti-inflammatory properties of Collagen Type 0 can impact the healing of a cranial (calvarial) wound in rats[4]. It also looked at the potential of Collagen Type 0 for bone regeneration. Collagen Type 0 was implanted into rats with a calvarial defect. A control group was also examined, in which no collagen was implanted. M1 and M2 macrophage balance, as well as bone formation, was measured after 60 days.

M1 and M2 macrophage balance

After 60 days, **Collagen Type 0** induced a majority M2 macrophage response, with more than 5-times as many M2 macrophages as when **no collagen** was implanted ($p<0.001$) (Fig. 8). There was a predominantly M1 macrophage response when no collagen was implanted.

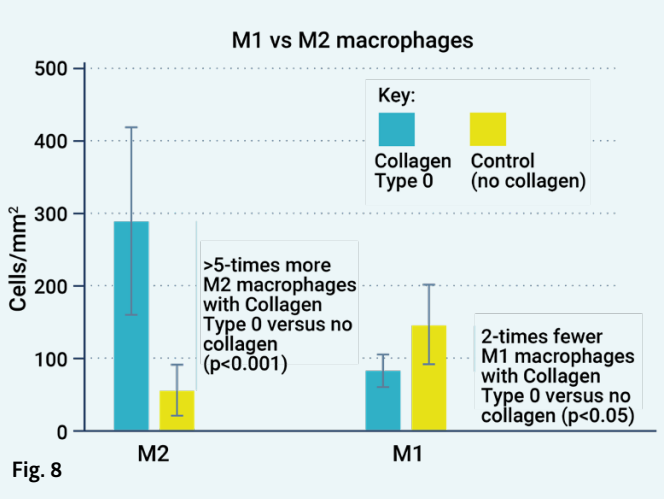


Fig. 8

Bone formation

When examining bone formation, there was >45% bone growth in the **Collagen Type 0** group with a significantly lower percentage (9%) in the group with **no collagen** implanted ($p<0.001$) (Fig. 9).

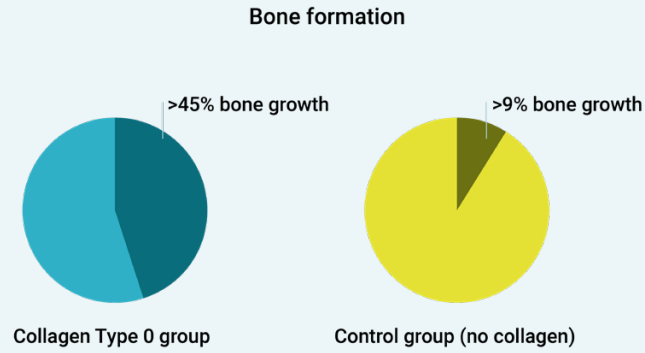


Fig. 9

Key takeaway: Collagen Type 0 shows tissue regeneration properties, associated with a predominantly M2 macrophage response.

Study 3) Wound healing ability in an impaired wound

In this *in vivo* study, we looked at whether Collagen Type 0 can close and heal full-thickness excisional wounds where there is impaired healing[5]. A diabetic mouse model was used to simulate impaired wound healing. As well as Collagen Type 0, we also looked at an alternative treatment, a bovine collagen predicate wound device (granulated bovine type I collagen combined with glycosaminoglycans), alongside non-treated controls. The wounds were monitored visually and through histological analysis to compare the progression of healing.

Wound closure

Wounds treated with **Collagen Type 0** reached 80% closure faster than those **not treated** (<20 days versus 44 days) (Fig. 10). The **bovine collagen predicate wound device** gave similar initial results. However, it gave large variability and poor performance in the final 20% of closure, with many wounds failing to close. **Collagen Type 0** resulted in superior uniformity and complete wound closure.

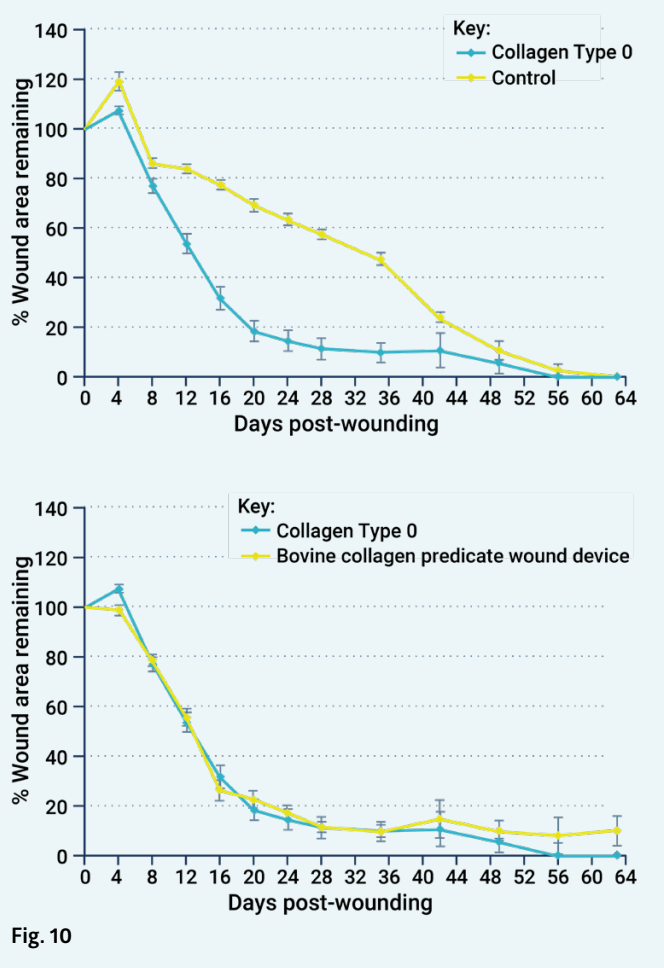


Fig. 10

Wound epithelialization and granulation

Collagen Type 0 led to formation of new epithelium similar in depth to normal skin; granulation of the wound bed (when tissue progresses from inflamed to being restored in wound healing) developed rapidly with both Collagen Type 0 and the bovine collagen predicate wound device but was increased with the latter.

Histology

Collagen Type 0: robust, organized and mature and well vascularized tissue with highly aligned cells and little evidence of unfavourable inflammatory reactions

Bovine collagen predicate wound device: weak, disorganized and poorly developed tissue

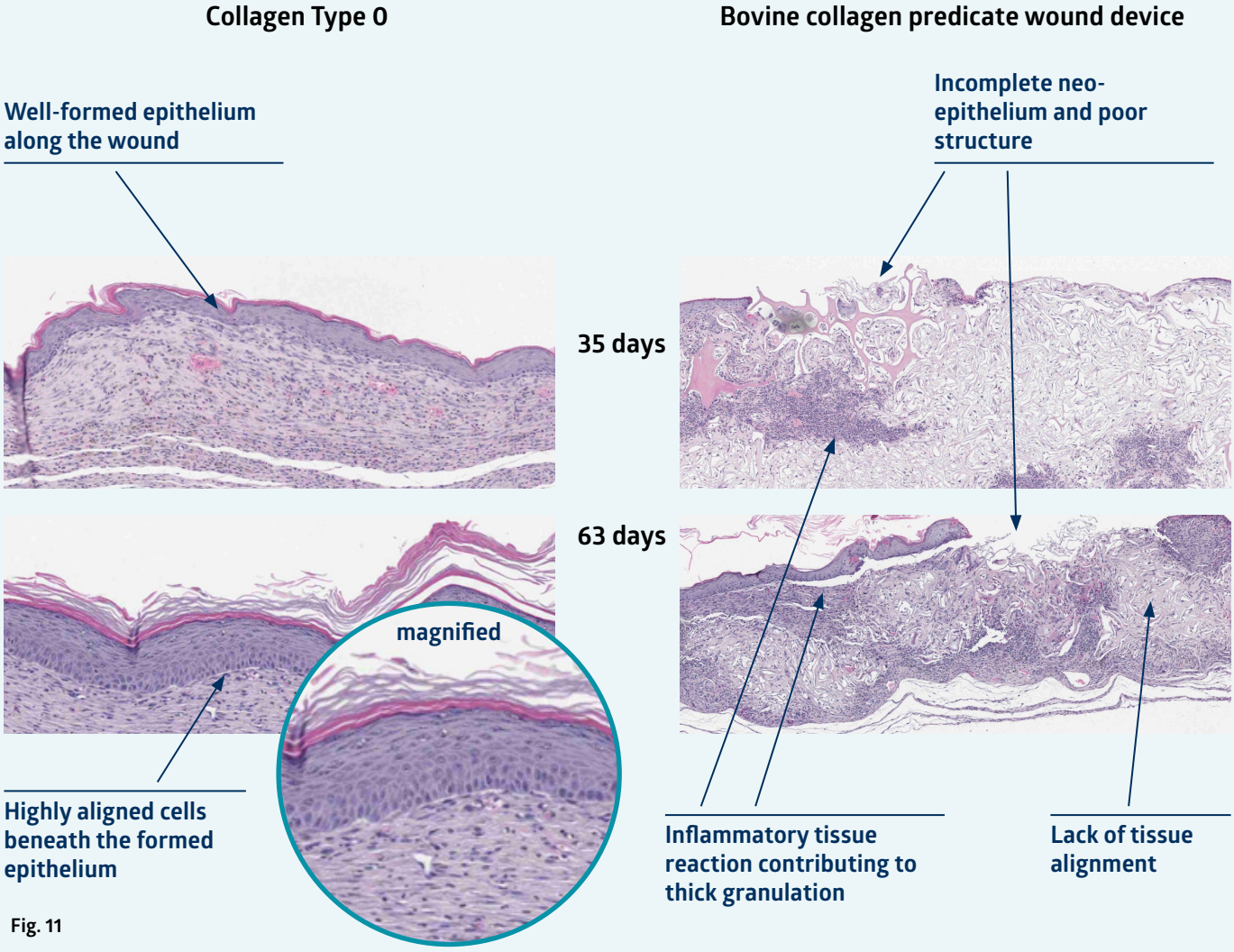


Fig. 11

Clinical applications

Due to the unique properties of Collagen Type 0 described, we are investigating its potential in several clinical areas. Firstly, and as described in this paper, in the treatment of chronic wounds (e.g. diabetic foot ulcers), to provide enhanced healing, prevent infections and protect patients from further complications (e.g. amputations). Secondly, we are looking at how the unique biocompatible and regenerative properties of Collagen Type 0 can be utilized for other medical treatments. These include in the treatment of osteoarthritis with cartilage repair strategies and for the treatment of vocal cord paralysis.

"Collagen Type 0, with its unique properties, provides a novel way of healing that is far more organized, with less scarring. This has been seen in several of the current applications, which are clinically very exciting. These applications alone could offer millions of patients a much better healing and recovery."

Dr Andy Weymann, Executive Medical Advisor, Jellagen

"Collagen Type 0 offers a paradigm shift in collagen chemistry. My intuition as a marine biotechnologist, was that if I explore a novel biology, this would also provide a novel chemistry. This goal led me to establish Jellagen, which is now bearing fruit given the exciting data Jellagen and our partners have generated. We have shown a clear differentiation from other collagen types and the potential future clinical benefit of Collagen Type 0 to patients. We are excited to forge future partnerships, in order to realise this potential for patients."

Professor Andrew Mearns Spragg, Founder and Chief Scientific Officer, Jellagen

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