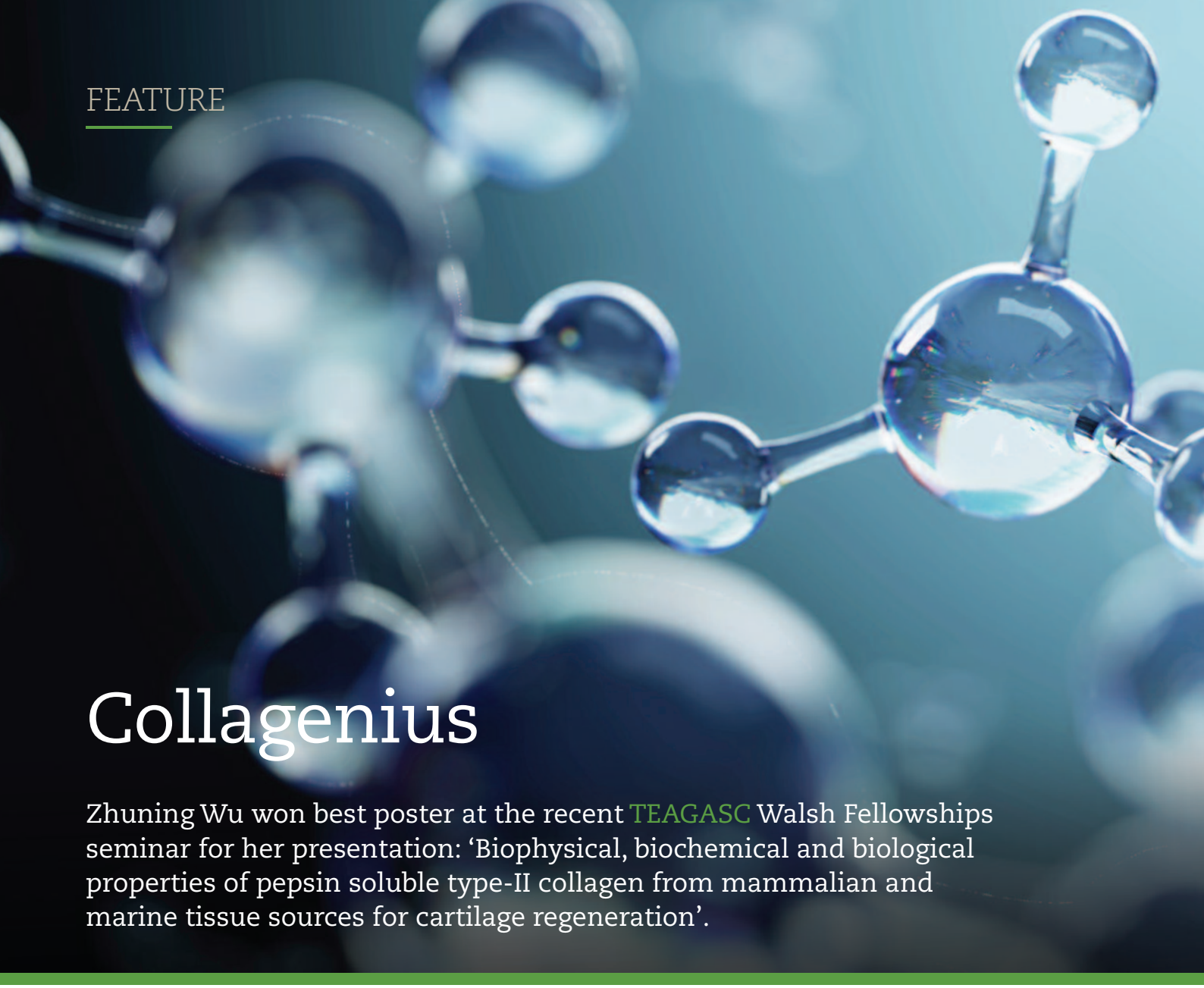

# Collagenius

Zhuning Wu won best poster at the recent TEAGASC Walsh Fellowships seminar for her presentation: 'Biophysical, biochemical and biological properties of pepsin soluble type-II collagen from mammalian and marine tissue sources for cartilage regeneration'.

## Introduction

Investigating high-value, biomedical opportunities for meat co-products will contribute to the sustainability of the Irish meat processing sector. These co-products are essentially the non-meat components arising from meat processing (e.g., offal, cartilage) and are often rich sources for proteins, with applications in a variety of fields including tissue engineering. Tissue engineering involves generating bioactive scaffolds and combining them with cells with a view to producing replacements for damaged tissue. The worldwide market for tissue engineering is expected to grow from $7bn in 2016 to over $16bn by 2023, with a compound annual growth rate (CAGR) of 13.2%.

Articular cartilage is a layer of smooth, white tissue that covers the ends of bones where they meet to form the joint. Healthy articular cartilage helps joints to maintain flexibility with a low frictional coefficient. Since articular cartilage is a low metabolic tissue, devoid of blood vessels, lymphatics and nerves, cartilage generation following trauma and degeneration presents a difficult clinical issue. Current treatments include multiple drilling, abrasion arthroplasty, mosaicplasty, and autogenous and allogeneic chondrocyte transplantation. Advances in tissue engineering provide new choice for articular cartilage regeneration.

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## Predominant component

The extracellular matrix (ECM) is a complex of cross-linked proteins and macromolecules, which serves as a scaffolding or supporting matrix for cells in tissues and organs; in cartilage these cells are called chondrocytes. In addition to its structural role, it plays a key function in cellular communications and regulation, providing biophysical, biochemical and biological cues that are required for cell and tissue homeostasis. Cell adhesion (the process by which cells interact with each other or attach to a surface or substrate), proliferation and differentiation, or specialisation, are common functions. Collagen is the predominant component of the extracellular matrix in various connective tissues, and makes up 25% to 35% of the whole protein content in the body. A total of 29 types have been identified to date, with type II playing a particular supporting role in cartilagenous tissue, and is the major component of articular cartilage in the knee joint. From a tissue-engineering perspective, collagen possesses excellent

tissue compatibility, non-toxicity, and controllable biodegradation. In addition, its degradation products are absorbed easily without giving rise to an inflammation in the body. Collagen-based medical devices have been growing in value over the past decades in the tissue engineering field, providing treatment for focal defects of articular cartilage and a 3D matrix to support generation of chondrocytes from adipose-derived stem cells.

Therefore, the potential of an implantable type-II collagen sponge as a cell carrier for cartilage regeneration is being investigated in this project. We propose to isolate and characterise type-II collagen from porcine cartilage and cartilaginous fish through pepsin digestion, and test its usefulness in cartilage regeneration. A number of tests need to be carried out on the collagen to test its suitability for this use. These include physical assessments of the protein and also testing of how the collagen will function or react when implanted as a collagen sponge.

Using a variety of sources, namely male and female porcine trachea, auricular, articular cartilage and cartilaginous fish, collagen was extracted through acid-pepsin digestion at 4°C. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed to assess the purity of the extracted collagen solution, and to uncover the number and size of collagen chains. Results confirmed the purity and molecular weight of collagen polypeptide chains, as compared to commercially available collagen. High purity type-II collagen was obtained from both male and female articular cartilage and cartilaginous fish material.

## Fabricating sponges

Collagen sponges were fabricated by dissolving the material in acetic acid and freeze-drying at -80°C. 4-arm polyethylene glycol (PEG) succinimidyl glutarate (4SG, Mw 10,000) was used as a crosslinking agent to fabricate collagen sponges. The structure of collagen sponges was determined by scanning electron microscopy (SEM) and the images obtained verified that the necessary homogenous porous structure was obtained. The porous structure allowed cells to attach and grow in the three-dimensional scaffold.

Thermal stability was tested by differential scanning calorimetry (DSC). Thermal stability reveals the integrity of collagen under non-crosslinked and crosslinked conditions, and the stability of potential collagen biomaterials at body temperature. Higher denaturation temperature of collagen indicates higher thermal stability, which is a critical characteristic of collagen-based medical devices. The thermal transition curves displayed similar denaturation temperatures of extracted non-crosslinked collagen samples, while the crosslinker significantly increased thermal stability, indicating an improvement in denaturation temperature of collagen biomaterials.

Enzyme degradation assays demonstrated the degradation profile of collagen biomaterials and their resistance to enzymatic digestion by collagenase. Collagen isolated from male and female articular cartilage exhibited relatively higher resistance against enzymatic degradation compared to male and female tracheal and auricular cartilage groups. A significant increase against enzymatic degradation can be observed in all crosslinked groups.

To test the biological properties of collagen sponges, i.e., how cells proliferate and differentiate in collagen sponges, human adipose-derived stem cells were seeded onto collagen sponges and cultured for 21 days. All groups of collagen sponges supported the proliferation and chondrogenic differentiation of human adipose-derived stem cells. Immunocytochemistry was carried out to visualise the expression of chondrogenic-specific protein using antibodies capable of binding to the protein of interest. To characterise the gene expression as a consequence of chondrogenic differentiation, reverse transcription polymerase chain reaction (RT-PCR) was performed to detect mRNA expression of chondrogenic marker genes. Results indicated that collagen sponges have potential to regenerate cartilage-like scaffolds. An upregulation of chondrogenic markers was evident. This research demonstrates that porcine articular cartilage and fish cartilage yield high-purity type-II collagen, which supports high-value biomedical applications for the meat processing sector in Ireland.

## Acknowledgements

Zhuning Wu is in receipt of a Teagasc Walsh Fellowship. This work forms part of the ReValueProtein Research Project (Grant Award No. 11/F/043) supported by the Department of Agriculture, Food and the Marine (DAFM), under the National Development Plan 2007-2013 funded by the Irish Government. This work is also funded by Science Foundation Ireland (SFI) (Grant Number: 13/RC/2073). The authors would like to thank Prof. Skoufos and Prof. Tzora for providing the raw materials assessed in this work.

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