Strong, native & highly biocompatible – introducing next generation collagen fibers for the development of novel therapies in regenerative medicine

Discover Viscofan BioEngineering's collagen in R&D and medical quality for implants and coatings of medical devices

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Introduction

The requirements on biomaterials for new therapies in regenerative medicine are huge and – to make matters worse – variable. They have to be biocompatible, allow vascularization and have good handling capabilities. The implant should stay at the desired place and – if cells are applied – provide an easy to handle, cell-friendly support that adheres tightly to the tissue. Depending on the application, requirements with regard to elasticity, tear-resistance and biodegradability can be quite different.

Collagen offers ideal features as biomaterial for regenerative medicine

FIG. 1: *The Collagen Cell Carrier® (CCC) enables transfer of cultured cell layers for analyses or implantation*

Collagen is with ca. 25% the most abundant protein in the mammalian body. It represents the main structural protein of the extracellular matrix, a protein scaffold conveying structure and stability to tissues, offering sites for cells to attach to and also influencing and supporting the adherent cells.

As it is also highly conserved in the animal kingdom it is universally usable as a matrix for the culture of cells and importantly exhibits good biocompatibility *in vivo*. Therefore, it has best prerequisites to be used as a scaffold supporting tissue regeneration.

Most collagen products lack mechanical strength and nativeness

In conventional production collagen is extracted in a solubilized form by acids or enzyme treatment. The resulting subunits – the triple helical tropocollagens – have to be re-organized *in vitro* yielding only fibrils and thin fibers that merely qualify to build up hydrogels or thin coatings. These scaffolds lack mechanical stability and the natural structure of the complex fibers.

Other scaffolds produced by decellularization of collagen-rich tissues leaving only the collagen scaffold, bear risks of residual other proteins or cell fragments with high immunogenic potential and RNA or DNA contamination. The required intensive purification process affects the stability of the scaffold as well.

To intensify the stability of those biomaterials, chemicals like glutaraldehyde are used that insert crosslinks between neighboring protein chains. However, those agents are highly toxic and the extra crosslinks compromise the natural structure of the collagen fibers and are therefore antagonistic to their biocompatibility and biodegradability.

Viscofan BioEngineering has developed a process to utilize the inherent strength of collagen fibers and combine it with their other positive features.

A proprietary collagen production process makes all the difference: Combining biocompatibility and strength in native collagen fiber preparations

FIG. 2: *Viscolma® mass of insoluble collagen fibers*

FIG. 3: *gently extracted collagen type I fibers (mean length: 1380 µm, mean width: 20,5 µm)*

Viscofan BioEngineering builds on the expertise of 85 years in collagen extraction and processing at industrial scale. The manufacturing process aims at preserving the long, intact collagen type I fibers from bovine dermis in their natural form, at the same time ensuring high purity.

From the yielding viscous fiber mass Viscolma® (FIG. 2) we customize cell culture scaffolds and medical implants with unique features as new tools for regenerative medicine.

Membranes and mass for a broad range of medical applications

A thin and strong collagen membrane as natural support to culture, analyze or implant cells

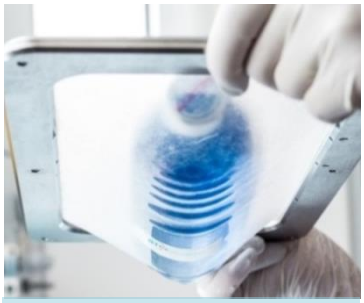

FIG. 4: *Wet CCC membrane (20 µm thin) carrying 500 ml bottle*

By flat extrusion of our fibrous mass Viscolma®, membranes down to 20 µm in thickness (e.g. the Collagen Cell Carrier® (CCC), FIG. 12) are formed, the compact fiber network conveying remarkable strength to the material without the need of extra chemical cross-linking (FIG. 3). The natural cell matrix made of native fibers is especially beneficial if cell transfer and implantation is intended.

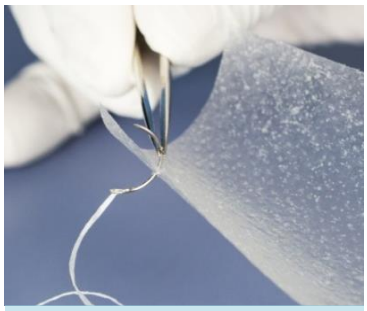

FIG. 5: *Suturable CCC membrane to fix implanted cells*

For this purpose, after cells have formed a robust cell-matrix-complex on the CCC membrane, the complex can be removed with forceps (FIG. 1) and sutured (FIG. 4, 5) to the recipient tissue. Its high elasticity makes it ideal as a patch to fix therapeutic cells in close proximity to damaged tissue[1,2] (Fig. 5 and see below).

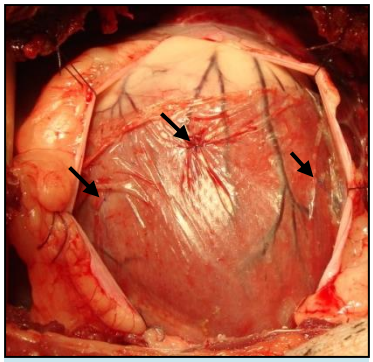

FIG. 6: *ADSC-seeded CCC sutured (➔) to minipig heart[2]*

In several independent labs the high biocompatibility of the Collagen Cell Carrier® has been confirmed in animal studies of various disease models. Also, the usability as a scaffold for the culture of more than 30 primary cell types, stem cells and cell lines has been tested including building up of stratified epidermal[3] and urothelial[4] tissues.

For various medical applications aimed at by our partners we develop membranes tailored to need regarding e.g. thickness, elasticity, permeability, degradation time, tear-resistance etc.

Novel and unique collagen material for coatings & development of new scaffolds

The high quality fibers of the collagen mass Viscolma® (up to 15% collagen fiber content in water) make it highly suitable as a biocompatible coating suspension for medical devices like e.g. stents or vessel prostheses. Beyond that, representing a novel and unique collagen material on the biomedical market, Viscolma® offers the chance to develop completely new scaffolds to improve therapies or address diseases that lack satisfying treatment to date.

Proprietary manufacturing in medical quality – *Made in Germany*

FIG. 7: *Production facility for medical grade collagen*

To facilitate a cost-efficient and fast bench-to-bedside transfer of novel collagen-based therapies we offer many of our products in R&D quality as well as in medical grade. A medical grade production facility has been inaugurated in 2017 for this purpose at our center of excellence for collagen in Weinheim, Germany. The proprietary technology was individually adapted to meet the regulations of the Good Manufacturing Practice (GMP) as were all the processes in our clean rooms down to ISO 5 (FIG. 6). Our certifications include DIN EN ISO 9001, ASTM F2212-02, DIN EN ISO 13485 (in progress) and we source the bovine hides for medical products from New Zealand, where BSE risk is negligible.

Table: Viscofan BioEngineering's collagen products at a glance

PRODUCT	APPLICATIONS	PROPERTIES	BENEFITS
Viscolma® collagen mass Starting material for our membranes	• **Development of biomedical scaffolds** • **Coating of medical devices**	• Viscous mass of insoluble collagen type I fibers (up to 15% in water) • Collagen fibers in highly preserved state: long, strong and native • Ultrapure • Products available in R&D and medical quality	• Unique fiber characteristics enable development of novel scaffolds • Natural environment for cells • Universally usable in different species and tissues => transferrable from model to model to patient • Easy and cost-efficient bench-to-bedside transfer
Collagen Cell Carrier® membrane (CCC)	**Cell-based therapies** (carrier for implantation of adhered cell layers)	• Pure collagen membrane with high mechanical strength yet low thickness (20 µm). No extra chemical crosslinking • Support for intact cell layers before, during & after implantation • Elastic, directly suturable and biodegradable *in vivo*[1,2] • Proven high biocompatibility in several independent animal studies [1,2,6]	• Improves cell survival & function by enabling continuous cell-cell and cell-matrix contact • Fixes implanted cells at desired place enhancing efficacy[1,2] • Reduces cell amount required for therapeutic effect[2] • Lowers experimental effort and costs • Enhances safety against risk of cells going astray
	• **Cell-based assays[3,4]** • Analyses of matrix-adhered cells	• Natural environment, supports high cell densities and stratification[1,2,3,5] • Removable from cell culture vessel with intact cell layer on top • minimal autofluorescence • resistant against contraction by fibroblasts • suitable for histologic analyses[3] • Chemically and thermostable from -175°C to +50°C	• Authentic cell performance • High significance of results • Easy handling and transfer of cultured cell layers • For analyses of cell-matrix interactions • Microscopy of fluorescence-labeled cells in high quality (even inverse epifluorescence)
Customizable collagen membranes	**Regenerative Medicine** with cells or cell-free (e.g. dura, wound repair…)	• Variable parameters: Mechanical strength, elasticity, thickness, biodegradation time, permeability, swelling properties • Suitable for addition of growth factors, antibiotics etc.	• Products tailored to customer needs, using 85 years of experience with collagen production at industrial scale • Quality control across the entire workflow ("Made in Germany")

Creating the ideal cell carrier: the booster for cell therapies

Collagen Cell Carrier® fixes ADSCs at infarcted heart tissue

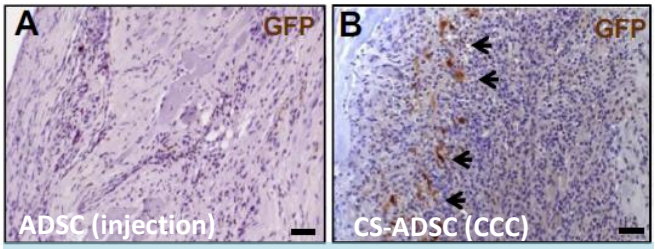

FIG. 8: *GFP-labeled ADSCs (→) in cardiac tissue 1 week post implantation in rats. A) „ADSC" by injection, B) „CS-ADSC" on CCC*[2]

The strong and biocompatible nature of our biomaterial makes it optimal for use in regenerative medicine. Together with the Spanish University of Navarra, our Collagen Cell Carrier® (CCC) membrane has been thoroughly investigated regarding the usability as a cardiac patch inducing heart muscle regeneration after an infarct in two animal models. Stem cells from adipose tissue (ADSCs) have been seeded on the CCC to form a multilayered cell-matrix-complex. The collagen membrane then was directly sutured to the scarred tissue. In the control, administration of cells by injection led to complete cell loss (FIG. 7A "ADSC") due to sweeping away by the bloodstream and by the deficiency to satisfy their contact-dependence. In contrast, among those cells that had been implanted adhered to the CCC, approx. 25% had grown into the tissue after one week (FIG. 7B "CS-ADSC")[1].

Significant improvement of cell engraftment and heart vascularization, elasticity & function

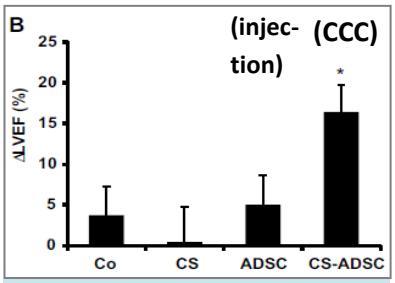

FIG. 9: *Left ventricular ejection fraction 4 months post implantation in rats*[2]

Three to four months after implantation, the heart pumping function had significantly improved, measured by 15% increased left ventricular ejection fraction in rats (FIG. 8) and minipigs. The researchers assign this improvement to several effects: Firstly, vascularization in the infarct-near zone was enhanced (FIG. 9), secondly the scarred and thus stiff tissue was reduced (FIG. 10), resulting in improved tissue elasticity that resembled now healthy heart. Additionally, there were hints that just the contact of the therapeutic cells with the collagen membrane changed their gene-expression in ways presumably supportive for their regenerative, paracrine impact on the damaged tissue. Finally, the CCC was degraded by the body within some weeks without notable immunoreaction (FIG. 11) [1,2].

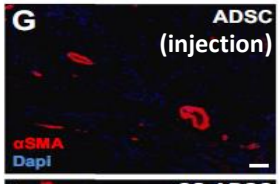

FIG. 10: *Analysis of vascularization by αSMA staining (red) in rat cardiac tissue*[2]

FIG. 11: *Analysis of fibrosis by Sirius Red staining in rat cardiac tissue*[2]

Conclusion

The Collagen Cell Carrier® enabled the success of this novel cell-based therapy by

1. **enabling the handling and fixing of the cells at the desired place,**
2. **supporting the cells to survive during and after the implantation process by forming a robust cell-biomatrix-complex beforehand,**
3. **potentially stimulating regenerative effects in implanted cells**

significantly enhancing the efficiency.

CCC enhances efficacy & safety and reduces effort & costs

The improved efficiency of the stem cell therapy caused by the CCC results in even more benefits: a relatively low number of cells is required to get a positive effect (5 x 10^7 cells in the minipig model)[2] which means a higher safety of the implant and less experimental effort and costs for the therapy.

Clinical phase I trial starts at end of 2018

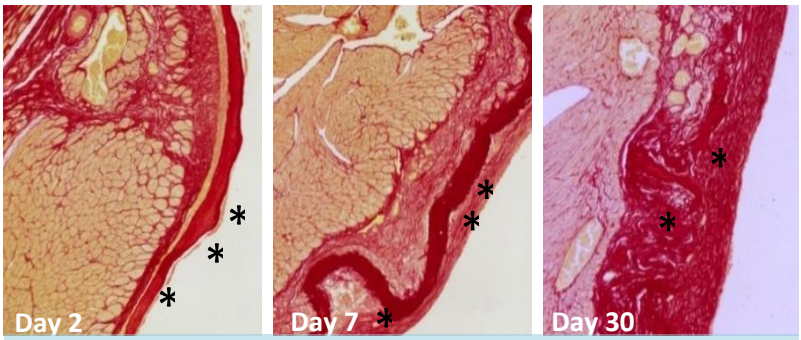

FIG. 12: *Absorption and degradation of CCC in rat cardiac tissue*[1]*. *: CCC*

Encouraged by the preclinical results for this Advanced Therapeutic Medicinal Product (ATMP), Viscofan Bioengineering is now working together with several institutions from industry, universities and hospitals to produce the medical grade end product of the CCC seeded with ADSCs and to evaluate it in patients. In 2016, the Spanish Ministry granted a budget of 1.26 million € to promote this project including clinical trials phase I which will begin at the end of 2018.

Additional activities include the development of collagen membranes for applications in dental medicine as well as for hernia surgery.

Be part of the medical progress: collaborators wanted!

FIG. 13: *CCC available in formats from 96 well to 100 x 150 mm*

Viscofan BioEngineering engages in promoting medical progress, e.g. ATMPs and other applications that benefit from our unique collagen products. We highly value a close partnership with customers and researchers interested in testing samples in their stride to advance medical research and development. This intimate interaction often gave rise to fruitful collaborations that support our vision to contribute to turning Europe into a point of reference in regenerative medicine.

Be part in the development of new collagen-based therapies to hitherto ill attended clinical conditions! We are open to all kinds of collaboration according to the needs of our counterparts: partnering for founded research projects, joint product development, OEM manufacturing, raw material provision, etc. Also companies interested in distribution of our products are welcome.

Benefit from 85 years of know-how in collagen production & processing and get in touch with us to obtain test samples and to discuss your needs:

contact@bio.viscofan.com

Get more information and browse our products on our website and shop:

www.viscofan-bioengineering.com

Literature

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Images

FIG. 1-5, 7, 13: © Viscofan BioEngineering
FIG. 6, 8-12: courtesy of University of Navarra

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