

## SCIENTIFIC ANALYSIS AND ENDORSEMENT OF NITTA CASINGS COLLAGEN GEL



12/26/2017

For Nitta Casings

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## About Dr. Silvia Minardi, PhD

Professional scientist with 10 years of research experience and successful delivery of biomedical technologies to address crucial clinical challenges, in orthopedics, general surgery, cancer, and more recently in diabetes and transplantation.

<https://www.linkedin.com/in/silviaminardiphd/>

**PhD Chemical Sciences**

**MS Industrial Biotechnology**

**BS Biotechnology**

### SKILLS

Biomedical Devices  
Collagen-based materials  
Personalized Therapeutics  
R&D  
cGMP Production

Orthopedics  
General Surgery  
Transplantation  
Diabetes  
Cancer

Quality Control  
Consulting  
Technical Support  
Project Leadership  
Invention Disclosures

### PROFESSIONAL APPOINTMENTS

Northwestern Medicine  
Feinberg School of Medicine

Department of Medicine  
(Chicago, IL)

Role: Scientist

Feinberg Center for Kidney  
Research and Therapeutics  
Pilot Feasibility Project

Northwestern University  
(Chicago, IL)

Role: Principal Investigator

Transplant Endowment Pilot  
Project

Northwestern Medicine  
(Chicago, IL)

Role: Principal Investigator

Institute of Science and  
Technology for Ceramics  
National Research Council  
of Italy (Faenza, Italy)

Role: Affiliate Member

Journal of Biomedical  
Microdevices

Role: Peer Reviewer

Journal of Translational  
Medicine

Role: Peer Reviewer

### MAIN PROFESSIONAL ACHIEVEMENTS

#### PUBLICATIONS AND PATENTS

30+ peer-reviewed scientific publications and books, 20+ presentations at national and international science conferences, and 1 US patent (Full list upon request).

#### HONORS AND AWARDS

15+ academic honors and awards, in the field of biomedical research (Full list upon request)  
Member of over 5 international scientific societies

## Background on collagen

Collagen is the most abundant structural protein of the extracellular matrix of connective tissues, and accounts for approximately 30% of the total protein content of mammals [1, 2]. Collagen plays a crucial role in tissue homeostasis, as well as pathogenesis and repair. It is paramount to cell migration, attachment and organization within tissues [3]. In particular, type I collagen molecules self-associate, and present D-periodic fibrils, which are the main component of skin, bone and tendons [4]. Type I collagen has become an extremely valuable material in research and medicine. Its high biocompatibility and versatility make it a material of choice in the design of biomedical devices. Collagen has been successfully used in a plethora of research and medical applications.

## Analysis of NITTA GEL

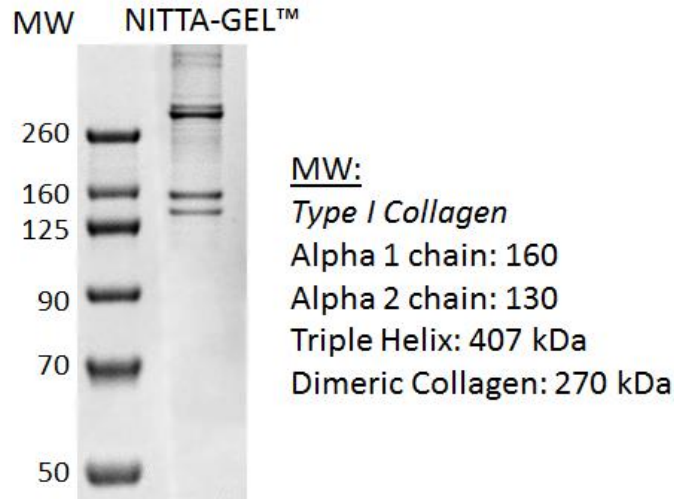
Summary table:

Characteristic/Requirement	Specifications
Species	bovine
Color	off-white
pH	2
Viscosity	~1,000,000 cP
Purity	≥95% within $\alpha$ , $\beta$ chains
Structure	Fibrillar (D-periods)

pH: the pH was measured with a pH-meter (Mettler Toledo).

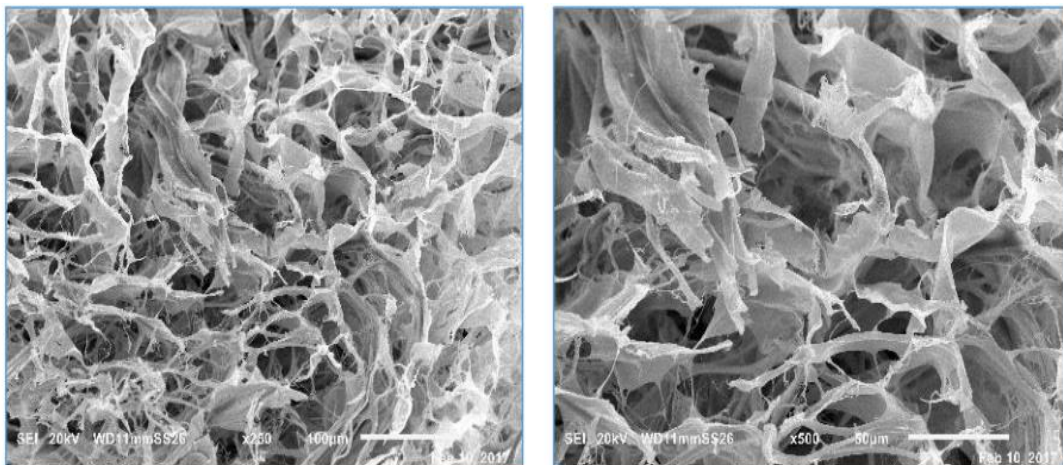
Viscosity: the viscosity of the gel was measured with a viscometer (Thermo Fisher)

Purity: The purity of NITTA-GEL was assessed by SDS-PAGE (Figure 1). The SDS-PAGE gel showed the typical bands of type I collagen, and assessed the high purity of the sample (≥95%).



**FIGURE 1 - SDS-PAGE (COOMASSIE BLUE STAINING) OF NITTA-GEL SHOWING THE TYPICAL PATTERNS OF BANDS OF TYPE I COLLAGEN.**

The micro-fibrillar structure of Nitta Casings type I collagen was characterized by scanning electron microscopy (SEM). The SEM micrographs are reported in figure 2, and revealed a highly fibrous architecture.



**FIGURE 2 - SCANNING ELECTRON MICROSCOPY MICROGRAPH OF NITTA GEL [MAGNIFICATION: 250X AND 500X, RESPECTIVELY].**

Finally, the nanostructure of single fibrils was evaluated by transmission electron microscopy (TEM) (Figure 3). As shown in the image, NITTA GEL presents the typical D-period of type I collagen fibrils of approximately 67 nm.



**FIGURE 3 - TRANSMISSION ELECTRON MICROSCOPY MICROGRAPH OF NITTA-GEL TYPE I COLLAGEN.**  
[MAGNIFICATION: 10000X]

## Conclusions – NITTA GEL

NITTA GEL consists of non-cross-linked, native type I collagen, produced from BSE-free bovine herds.

In NITTA GEL, type I collagen is solubilized and the fibrillogenesis can be easily initiated by pH-driven self-assembly, to obtain the desired degree of fibrillation. This raw collagen gel is ready to be used for the fabrication of any bioengineering devices. Its quality is superior than the selected research-grade competitor product (Sigma Aldrich), and comparable to the selected medical-grade product (Collagen Solutions).

Excellent biocompatibility and bioactivity was demonstrated, both *in vitro* and *in vivo* experiments reported in international peer-reviewed research studies [5-9].

## Endorsement data

A weighted decision matrix was used to endorse the quality of the type I collagen of NITTA GEL, with respect to the main research-grade and medical-grade competitor raw collagen products. This basic decision matrix established a set of criteria which were considered relevant for the biomedical field (e.g. purity, bioactivity). The criteria were ranked (weighted) from 1 to 5, as reported in the following table:

<b>Decision Model</b>	
<b>Criterion</b>	<b>Weight</b>
<b>Purity</b>	5
<b>Fibrillogenesis</b>	5
<b>Bioactivity</b>	5
<b>BSE-free</b>	4
<b>Endotoxin</b>	3
<b>Sterility</b>	2
<b>Certifications</b>	2
<b>Price</b>	1

The highest weight was assigned to purity and fibrillogenesis, which indicate the preservation of the collagen fibers. Fibers' integrity and the ability to self-assemble is a crucial characteristic of type I collagen, paramount for its function and bioactivity. Therefore, the criterion "bioactivity" was also ranked with a "5". These criteria indicate the ability of the manufacturer to preserve the intrinsic chemical, structural and biological properties of type I collagen, during manufacturing.

The source of the raw materials (BSE-free), its endotoxin content, sterility, certifications provided by the manufacturer and price were assigned decreasing weight scores. These criteria can be adjusted in quality, depending the manufacturer's needs.

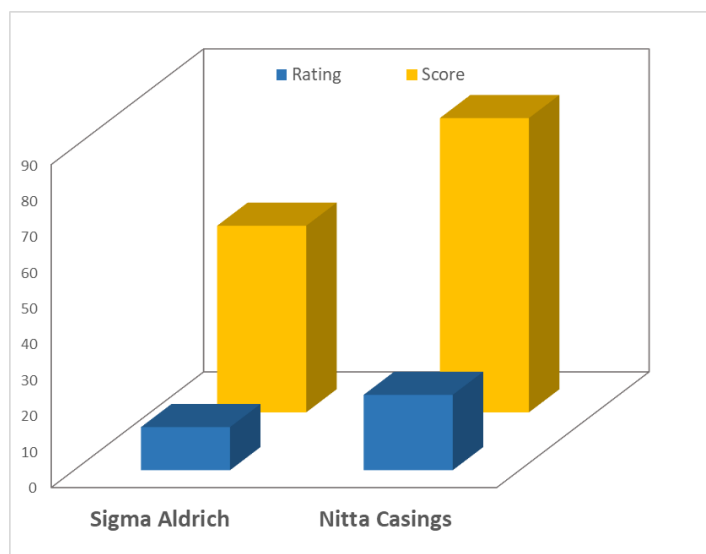
Each criterion was scored with a rating system ranging from 0 to 4, as follow:

<b>Rating</b>	<b>Description</b>
0	Bad
1	Poor
2	Avarage
3	Good
4	Excellent

Weighted scores were calculated accordingly, and a “total” score was calculated per each sample analyzed with the matrix. The results of the decision matrix are reported below.

Research-grade comparison:

- Research-grade Sigma Aldrich bovine collagen (product# C9879)
- NITTA GEL

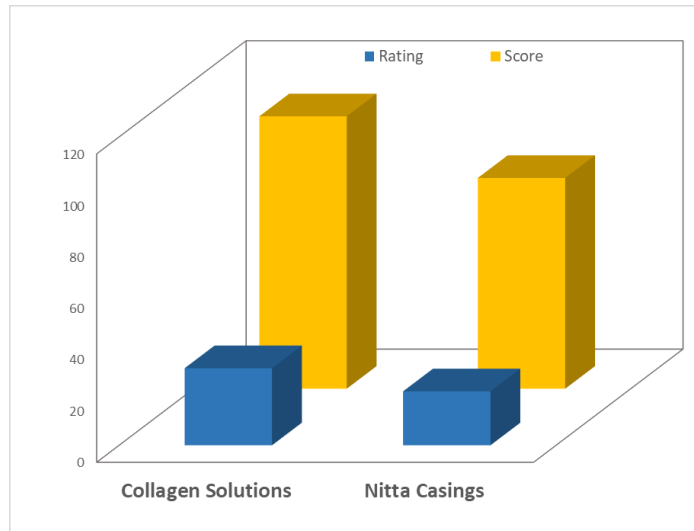


Decision Model		Sigma Aldrich Research Grade		Nitta Casings Research Grade	
Criterion	Weight	Rating	Score	Rating	Score
Purity	5	4	20	4	20
Fibrillogenesis	5	2	10	4	20
Bioactivity	5	4	20	4	20
BSE-free	4	0	0	4	16
Endotoxin	3	0	0	0	0
Sterility	2	0	0	0	0
Certifications	2	0	0	1	2
Price	1	2	2	4	4
<b>Total</b>	<b>27</b>	<b>12</b>	<b>52</b>	<b>21</b>	<b>82</b>



Medical-grade comparison:

- Medical-grade Collagen Solutions bovine collagen (product# FS28001)
- NITTA GEL



Decision Model		Collagen Solutions Medical Grade		Nitta Casings Research Grade	
Criterion	Weight	Rating	Score	Rating	Score
Purity	5	4	20	4	20
Fibrillogenesis	5	4	20	4	20
Bioactivity	5	4	20	4	20
BSE-free	4	4	16	4	16
Endotoxin	3	4	12	0	0
Sterility	2	4	8	0	0
Certifications	2	4	8	1	2
Price	1	2	2	4	4
<b>Total</b>	<b>27</b>	<b>30</b>	<b>106</b>	<b>21</b>	<b>82</b>

Results:

NITTA GEL was found to be overall superior to its main research-grade competitor.

It was also found to be comparable to the medical-grade collagen for purity, fibrillogenesis, bioactivity and BSE-free standards. In addition, it demonstrated superior in price. cGMP, endotoxin-free and sterility certifications are currently not available for NITTA GEL. Thus, NITTA GEL did not meet the same quality standards for sterility of the medical-grade competitor type I collagen product.

## Supporting References

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- [2] J.F. Bateman, S.R. Lamande, J.A. Ramshaw, Collagen superfamily, *Extracellular matrix 2* (1996) 22-67.
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- [5] S. Minardi, B. Corradetti, F. Taraballi, M. Sandri, J. Van Eps, F.J. Cabrera, B.K. Weiner, A. Tampieri, E. Tasciotti, Evaluation of the osteoinductive potential of a bio-inspired scaffold mimicking the osteogenic niche for bone augmentation, *Biomaterials* 62 (2015) 128-37.
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