REVIEW

VOLUME 73 | ISSUE 3 | JULY - SEPTEMBER 2022

Application of collagen-based scaffolds for the treatment of spinal cord injuries in animal models. A literature update.

Zachariou D[1], Vlamis I[2]
3rd Orthopaedic Dept, National and Kapodistrian University of Athens, Greece

ABSTRACT

This review compiles newer bibliographical data with regards to the application of collagen scaffolds for the purposes of treatment of Spinal cord injury (SCI) in animal models. SCI is regarded as one of the most devastating central nervous system (CNS) injuries, exhibiting an alarmingly rising incidence rate, indirectly connected with the expansion of global economy. The consequences of SCI are multidimensional: SCI injuries may result in permanent voluntary motor disfunction and loss of sensation, while incurring heavy economic and psychological burden as part of the treatment. Thus, it is of great importance that effective and suitable SCI treatment strategies are developed. Collagen-based scaffolds application is one of the most promising methods of SCI treatment. They come in a variety of forms, including hydrogel, sponge or guidance conduit serving as an instrument to administer therapeutic drugs and proteins to the SCI site. A number of relevant studies have been carried out fairly recently, exclusively using carefully selected animals that resemble human pathophysiology and surgical outcomes, without incurring cost-related, ethical or regulatory limitations. In mouse, rat and canine models having mainly undergone transection and hemisection, the stump connection, along with transplanted cell differentiation, elimination of glial scar, increased neuronal growth, decreased collagen deposition, behavioural recovery, improved electrophysiology and enhanced axonal regeneration are evident.

KEYWORDS: Spinal Cord Injury, Animal Model, Collagen-Based Scaffold, Regenerative Medicine, Tissue Engineering

Introduction

Spinal cord injury (SCI) is regarded as one of the most devastating central nervous system (CNS) injuries, exhibiting an alarmingly rising incidence rate, indirectly associated with the expansion of global economy [1-5]. The consequences of SCI are multidimensional: SCI injuries may result in permanent voluntary motor disfunction and loss of sensation, while incurring heavy economic and psychological burden []. Thus, it is of great importance that

CORRESPONDING AUTHOR, GUARANTOR

Dimitrios Zachariou, Postgraduate training program,
National and Kapodistrian University of Athens, KAT Hospital,

effective and suitable SCI treatment strategies are developed [1].

More specifically, SCI causes neurological disabilities as the CNS central axons or nerve fibres, more often than not, fail to regenerate [4,6] owing to chronic inflammatory response, demyelination and increased levels of proteoglycans [6]. In mammalian CNS, the leading cause behind the limitation of central axons to regenerate is glial scar formation, which inhibits axonal remodeling and regrowth [4,7]. Healthy glia cells are known to support neuronal function as well as signal transmission [8]. However, when SCI occurs, , the borders of SCI lesion are separated from healthy tissue by a glial scar densely populated by newly hypertrophic and proliferating astrocytes [4]. The mechanical trauma may well further progress, owing to recruitment facilitation and non-resident cell infiltration, both of which are known to jeopardise regeneration of myelin sheath and function of neurons, with glial scar constituting a physical and molecular barrier to the development of CNS axons [8,9].

Consequently, the majority of therapeutic strategies developed in recent years focus on eliminating the post-SCI inhibitors of regeneration. The strategies provide support and guidance towards regeneration of affected neurons by the application – among others – of neural scaffolds onto the SCI site [1]. The advanced tissue engineering technology has paved the way toward SCI treatment [3,10,11]. The extracellular matrices (ECM) allow living cell inoculation, growth and differentiation, promoting the regeneration of axons and fibers. The matrices are co-cultured with cells and are then transplanted in the SCI area [4]. This way, the extracellular matrix of the spinal cord can be successfully mimicked, as scaffolds are rich in glycosaminoglycan, a gap-filling polysaccharide of staunch structure [4]. However, excessive amounts thereof contribute to the uncontrollable development of extensive and grave glial scar. The most suitable solution to combat this issue shall be collagen [4].

Taking the above into consideration, there is indeed a great deal of attention regarding the characteristics of scaffolds, especially of the biomaterials these are made of. Wang et al. [12] stress the importance of the biocompatibility for cells, apposite porosity topography and permeability of scaffold materials. As aforementioned, collagen-based scaffolds are a popular choice when it comes to biomaterials used for SCI treatment purposes. Collagen is a protein found in abundance in the ECM, provoking minimal autoimmune response, promoting adhesion, proliferation, migration and differentiation of cells [13]. Collagen scaffolds come in a variety of forms, including hydrogel, sponge or guidance conduit serving as an instrument to administer therapeutic drugs and proteins to the SCI site [14].

This review constitutes a compilation of newer bibliographical data on collagen scaffolds, as applied for SCI treatment purposes in animal models, aiming to provide a fresh insight on the available bibliography.

Discussion

This review has been compiled of articles and other bibliographical sources available on databases of strictly academic, peer-reviewed content. The search terms have been: "Spinal cord Injury, Animal Models, Collagen, Scaffolds, Tissue Engineering, Biomaterials, Regeneration Medicine". The results were filtered so that they were not published before 2014. Pieces of research not referring directly to animal models, SCI experiments and collagen scaffolds were also disqualified (Table 1).

Pathophysiology of SCI

The spinal cord injury pathophysiology is often broadly categorized as either "acute impact" or "compression". Injury as a result of acute impact is essentially a spinal cord concussion. This mere concussion triggers a series of reactions localized in the grey matter, concluding to hemorrhagic necrosis. A grey matter hypoperfusion is usually the trigger of the series of events mentioned in this section. Occurring soon after the injury, reperfusion and increase in intracellular calcium are crucial for the injury outcome. The necrosis extent is analogous to the amount of initial force that caused the trauma, being also dependent on perfusion pressures, concomitant compression, pharmacological agents administration as well as blood flow. Mechanisms that

VOLUME 73 | ISSUE 3 | JULY - SEPTEMBER 2022

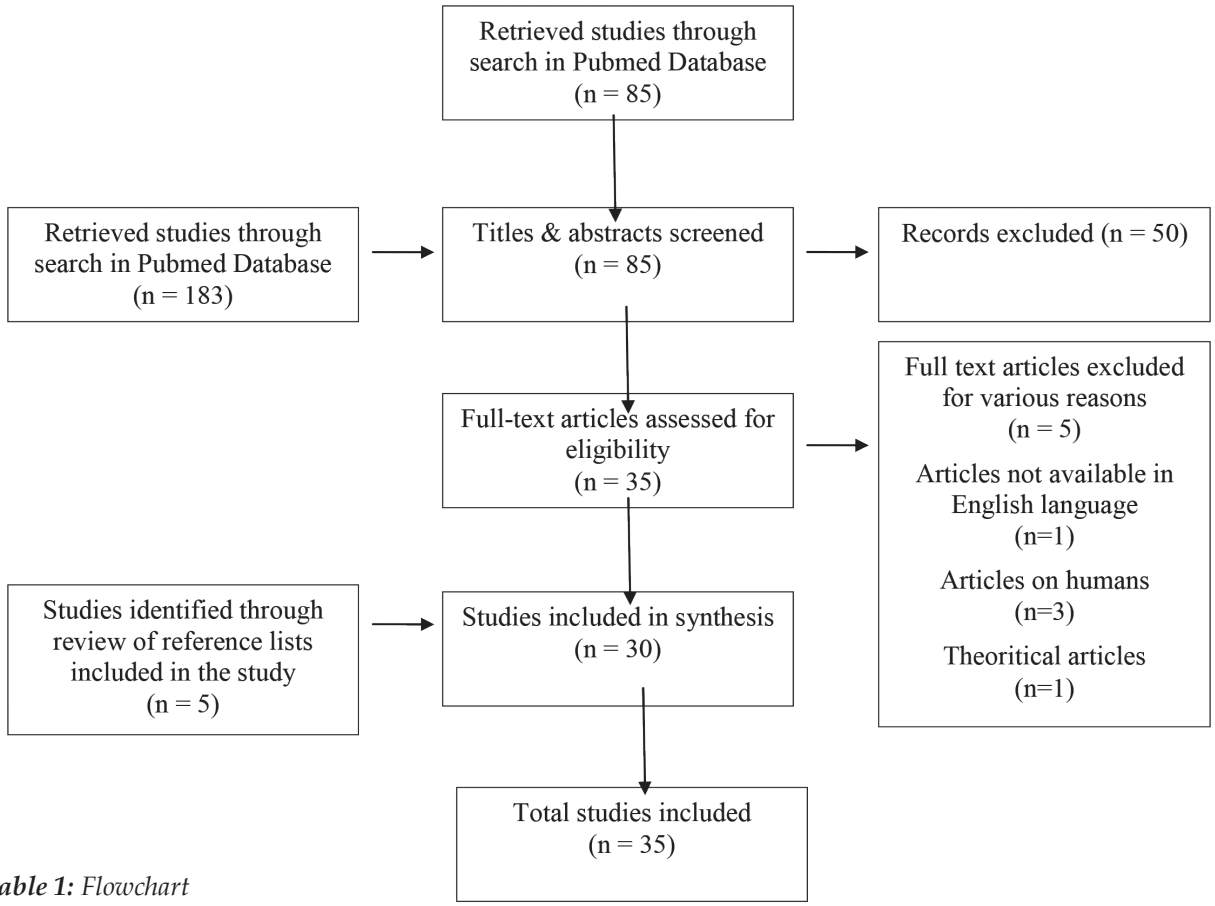

Table 1: *Flowchart*

take place in the initial stages of the injury should be targeted for a better prognosis. Injury as a result of spinal cord compression occurs upon impingement of the spinal cord by a mass, resulting in an increase of the parenchymal pressure. The tissue response is gliosis, demyelination, and axonal loss. This occurs in the white matter, whereas gray matter structures are preserved. Rapid or a critical degree of compression will result in collapse of the venous side of the microvasculature, resulting in vasogenic edema. Vasogenic edema exacerbates parenchymal pressure, and may lead to rapid progression of dysfunction. Treatment of compression should focus on removal of the offending mass.

Collagen Scaffolds for SCI Treatment

In line with the above, a number of Regeneration Medicine (RM) and Tissue Engineering (TE) studies prove the effectiveness of injected collagen hydrogels. Notably, Breenet et al. [14] examined injectable collagen hydrogels role in administering neurotrophin-3 into rat models that have undergone hemisection SCI. Others [15] discuss the positive results following the transplantation of collagen scaffolds loaded with stem cells in a mouse SCI model. In another study deploying canines with a "complete spinal cord transection, a linear ordered collagen scaffold was seeded with human mesenchymal placenta cells, demonstrating positive effects after transplantation [15]. Lastly, the team led by Jianwu Dai developed a collagen-based scaffold after ten years of research. It caused minimal side effects and exhibited increased therapeutic effectiveness. [16-18].

Li et al. [19-22] have also presented a series of outstanding pieces of research on regeneration and overcoming inhibitory factors after SCI. More specifically, they analyzed the delivery of proteins and drugs through scaffolds, to enhance post-SCI recov-

ery, mainly in animal models. Their oldest study mentioned in this review [19] refers to the construction of the CBD-EphA4LBD and CBD-PlexinB1LBD collagen-binding proteins, which help neutralize the ephrinB3 and sema4D molecules that inhibit neuronal regeneration. The proteins, administered with the use of collagen scaffolds, could promote outgrowth of neurities in vitro. Being immobilized by the scaffold itself, they were delivered by the transplantation of the latter into a rat that had sustained SCI, restoring locomotion.

In 2016, [20] the same group of scientists presented a porous collagen scaffold as means of neurotrophic protection, with seeded cerebellar granular neurons showing outgrowth in-vitro. Combined with Cyclic Adenosine Monophosphate (cAMP), the scaffold aided the repair of a completely transected spinal cord in a rat model.

In 2017, [21], Li et al reverted with another study, whereby they discussed the phenomenon of non-differentiation of endogenous Neural Stem Cells (NSCs) in rats with grave SCI. Cetuximab, a signaling antagonist, was administered via the implantation of Modified Linear Order Scaffolds (LOCS), increasing neurogenesis in lesions found in rats and, subsequently, dogs.

Most recently, Li et al [22] demonstrated that paclitaxel (PTX) reduced glial scarring attributed to SCI, by rescuing myelin-inhibited differentiation of NSCs. The cells were co-cultured with PTX and transplanted via a functional collagen scaffold into a complete T8 transection of spinal cord in a rat model. Improvement of sensation and locomotion was confirmed by Western Blot (WB) and mRNA-Seq results that showcased the ability of PTX to trigger neuronal differentiation via Wnt/β-catenin signaling pathway.

The effect of collagen-based scaffolds as a means of release of therapeutic substances was also discussed earlier by Snider et al [23], whereby the effectiveness was demonstrated using rat models to provide relevant evidence both during the acute and chronic SCI phase.

The importance of Animal Models in SCI studies

Sharif Al-Hoseini et al. [24], noticing the importance of using animal models in SCI studies, conducted a systematic review on "Animal Models of Spinal Cord Injury. The researchers categorized 2209 injuries according to level, outcome, animal species and purpose of study. Most of the reviewed studies examined drug effectiveness, while others simply observed pathophysiologic changes. Eighty one per cent of SCI sites involved the thoracic region, whilst contusion, transection and compression were the most common injury types induced. The majority (72,4%) of SCI assessments were conducted on rats, as the biological and behavioural outcomes, as well as the biomechanics and neuropathology of the rodents highly resemble those of humans. According to the study [24], rodents, in general such as mice or rats, are an optimal choice when it comes to preliminary SCI studies because of the low reproductory cost thereof and resemblance to human beings in terms of pathology and genomes. Cats are another popular choice in SCI studies, especially because of their larger size, compared to rodents, which allows easier surgical maneuvers. Another important preclinical model is the pig, which combines an intermediate size and greater resemblance to human physiology. Fish, lamprey and other vertebrates have also been deployed in novel SCI studies, owing to their unique regeneration capability. The study [24] continues to point out that the optimal choice for SCI studies would be the non-human primates and larger animals that actually represent human SCIs a lot better than other organisms. Notwithstanding the above, these primates are not ultimately deployed in such studies because of costly care, as well as regulatory and ethical considerations. As an alternative, canines can be studied in laboratory conditions after naturally-occuring SCI (e.g., due to accidents), causing less of ethical concern.

In 2021 [1], a systematic literature review has compiled a number of SCI studies conducted in animal models. Most importantly, as shown in Table 1, four studies [24-27] carried out between 2014 and 2016 have applied collagen-based scaffolds for the purposes of SCI treatment in mouse, rat and canine models. In these cases of transection and hemisection, stump connection, transplanted cell differen-

VOLUME 73 | ISSUE 3 | JULY - SEPTEMBER 2022

tiation, elimination of glial scar, increased neuronal growth, decreased collagen deposition, behavioural recovery, improved electrophysiology and enhanced axonal regeneration were noted.

Conclusions and perspectives

This review has aimed to compile the latest bibliographical data available with regards to the application of collagen scaffolds for the purposes of treatment of SCI in animal models. SCI is one of the most critical cases a patient and a surgeon may encounter, bearing significant economical and psychological implications, along with an increased rate during the recent years. Subsequently, novel ways of SCI treatment shall be developed.

Collagen-based scaffolds application is one of the most promising methods of SCI treatment. A number of relevant studies have been carried out fairly recently, exclusively using carefully selected animals that resemble human pathophysiology and surgical outcomes, without incurring cost-related, ethical or regulatory limitations. The results have been encouraging, with axonal regeneration, elimination of glial scar, as well as improved sensation and locomotion of animal models.

However, there is always room for improvement. May this review be the beginning of a new wave of research, suggesting minimally invasive, highly effective and biocompatible solutions that can actually improve the outcome and quality of life of every SCI patient.

REFERENCES

1. Litowczenko J, Woźniak-Budych MJ, Staszak K et al. Milestones and current achievements in development of multifunctional bioscaffolds for medical application. Bioactive Materials. 2021;6(8):2412-38
2. Silva R., Singh R., Sarker B et al. Hydrogel matrices based on elastin and alginate for tissue engineering applications. Int. J. Biol. Macromol. 2018
3. Furlan JC, Craven BC, Massicotte EM et al. Fehlings MG. Early versus delayed surgical decompression of spinal cord after traumatic cervical spinal cord injury: a cost-utility analysis. World neurosurgery. 2016;88:166-74
4. Yeh JZ, Wang DH, Cherng JH et al. Collagen-Based Scaffold for Promoting Neural Plasticity in a Rat Model of Spinal Cord Injury. Polymers. 2020;12(10):2245
5. Quraishe S., Forbes L.H., Andrews M.R. The extracellular environment of the cns: Influence onplasticity, sprouting, and axonal regeneration after spinal cord injury. Neural Plast.2018;2018:2952386
6. Dias DO, Kim H, Holl D et al. Reducing pericyte-derived scarring promotes recovery after spinal cord injury. Cell. 2018;173:153-65
7. Xu B., Park D., Ohtake Y. et al. Role of CSPG receptor LAR phosphatase in restricting axon regeneration after CNS injury. Neurobiol. Dis.2015;73:36-48
8. Orr M.B., Gensel J.C. Spinal cord injury scarring and inflammation: Therapies targeting glial andinflammatory responses. Neurotherapeutics. 2018;15:541-53.
9. Wang N, Xiao Z, Zhao Y, et al. Collagen scaffold combined with human umbilical cord-derived mesenchymal stem cells promote functional recovery after scar resection in rats with chronic spinal cord injury. Journal of tissue engineering and regenerative medicine. 2018;12(2):e1154-63.
10. Chen X., Zhao Y., Li X. Et al. Functional Multichannel Poly(Propylene Fumarate)-Collagen Scaffold with Collagen-Binding Neurotrophic Factor 3 Promotes Neural Regeneration After Transected Spinal Cord Injury. Adv. Healthc. Mater. 2018
11. Yao Q., Liu Y., Pan Y. Et al. One-pot porogen free method fabricated porous microsphere-aggregated 3D PCL scaffolds for bone tissue engineering. J. Biomed. Mater. Res. - Part B Appl. Biomater. 2020
12. Wang J., Zheng J., Zheng Q. et al. FGL-functionalized self-assembling nanofiber hydrogel as a scaffold for spinal cord-derived neural stem cells. Ma-

VOLUME 73 | ISSUE 3 | JULY - SEPTEMBER 2022

ter. Sci. Eng.C Mater. Biol. Appl. 2015;46:140-47.
13. Song R., Murphy M., Li C et al. Current development of biodegradable polymeric materials for biomedical applications. Drug Des. Devel. Ther. 2018
14. Breen BA, Kraskiewicz H, Ronan R et al. Therapeutic effect of neurotrophin-3 treatment in an injectable collagen scaffold following rat spinal cord hemisection injury. Acs Biomater Sci Eng. 2016;3:1287
15. Han S, Li X, Xiao Z, Dai J. Complete canine spinal cord transection model: a large animal model for the translational research of spinal cord regeneration. Science China Life Sciences. 2018;61(1):115-17
16. Xiao Z, Tang F, Tang J, et al. One-year clinical study of NeuroRegen scaffold implantation following scar resection in complete chronic spinal cord injury patients. Science China Life Sciences. 2016;59(7):647-55.
17. Xiao Z, Tang F, Zhao Y et al. Significant improvement of acute complete spinal cord injury patients diagnosed by a combined criterion implanted with neuroregen scaffolds and mesenchymal stem cells. Cell transplantation. 2018;27(6):907-15.
18. Zhao Y, Tang F, Xiao Z. et al. Clinical study of NeuroRegen scaffold combined with human mesenchymal stem cells for the repair of chronic complete spinal cord injury. Cell transplantation. 2017;26(5):891-900.
19. Li X, Han J, Zhao Y et al. Functionalized collagen scaffold neutralizing the myelin-inhibitory molecules promoted neurites outgrowth in vitro and facilitated spinal cord regeneration in vivo. ACS applied materials & interfaces. 2015;7(25):13960-71.
20. Li X, Han J, Zhao Y et al. Functionalized collagen scaffold implantation and cAMP administration collectively facilitate spinal cord regeneration. Acta biomaterialia. 2016;30:233-45.
21. Li X, Zhao Y, Cheng S et al. Cetuximab modified collagen scaffold directs neurogenesis of injury-activated endogenous neural stem cells for acute spinal cord injury repair. Biomaterials. 2017;137:73-86.
22. Li X, Fan C, Xiao Z et al. A collagen microchannel scaffold carrying paclitaxel-liposomes induces neuronal differentiation of neural stem cells through Wnt/β-catenin signaling for spinal cord injury repair, Biomaterials 2018, doi: 10.1016/j.biomaterials.2018.08.037.
23. Snider S, Cavalli A, Colombo F et al. A novel composite type I collagen scaffold with micropatterned porosity regulates the entrance of phagocytes in a severe model of spinal cord injury. J Biomed Mater Res Part B 2016:00B:000.
24. Sharif-Alhoseini M, Rahimi-Movaghar V. Animal models in traumatic spinal cord injury. Topics in Paraplegia. 2014:209-28. dx.doi.org/10.5772/57189
25. Sugai K, Nishimura S, Kato-Negishi M et al. Neural stem/progenitor cell-laden microfibers promote transplant survival in a mouse transected spinal cord injury model. Journal of neuroscience research. 2015;93(12):1826-38.
26. Han S, Wang B, Jin W et al. The collagen scaffold with collagen binding BDNF enhances functional recovery by facilitating peripheral nerve infiltrating and ingrowth in canine complete spinal cord transection. Spinal cord. 2014;52(12):867-73.
27. Han S, Xiao Z, Li X et al. Human placenta-derived mesenchymal stem cells loaded on linear ordered collagen scaffold improves functional recovery after completely transected spinal cord injury in canine. Science China Life Sciences. 2018;61(1):2-13.

READY - MADE CITATION

Zachariou D, Vlamis I. Application of collagen-based scaffolds for the treatment of spinal cord injuries in animal models. A literature update. *Acta Orthop Trauma Hell* 2022; 73(3): 254-259.