COMPARATIVE in vitro STUDY

Comparison of basic physicochemical parameters, USP parameters and mechanical and biological performance of regenerated and non-regenerated oxidized cellulose-based haemostats.

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Oxidized cellulose has been used in the health sector for many years as a localised haemostat. It can be advantageously applied wherever it is necessary to control capillary, venous and also minor arteriolar bleeding, when other conventional methods (such as ligation or cauterization) are not effective or practical. The benefits of oxidized cellulose are its biocompatibility, hypoallergenic and bactericidal properties and bio-resorbability.

Oxidized cellulose is prepared either by oxidation of premium quality extra-long staple cotton (oxidized non-regenerated cellulose, ONRC), or by oxidation of viscose (oxidized regenerated cellulose, ORC). Both types have very similar characteristics and practically the same indication. However, they may differ in some properties important for their clinical use.

Purpose of the comparative study

The aim of this study was to compare basic physicochemical and USP parameters of oxidized non-regenerated cellulose (*OKCEL®*, Synthesia, a.s., Czech Republic) versus oxidized regenerated cellulose (*SURGICEL®*, Ethicon, Inc., New Jersey, USA) and especially to evaluate their mechanical and biological performance by comparing parameters that are directly linked to their clinical use (such as adhesion, colouration in liquid, shape stability, positioning, absorbency and bio-resorbability).

Methodology

All parameters were tested under controlled laboratory conditions.

- **USP requirements** (loss on drying, ash content, nitrogen content, formaldehyde content, carboxyl group content and identity) were tested for *SURGICEL®* according to US Pharmacopoeia for oxidized regenerated cellulose and for *OKCEL®* according to verified analytical working instructions of Synthesia, a. s. that are based on US Pharmacopoeia methodologies.
- **Area density** (the mass per unit area expressed in grams per square centimetre) was calculated from a product sample weight and dimensions.
- **pH** was measured using a pH meter on a product sample converted into a solution.
- **Structure** was evaluated by using a scanning electron microscope (SEM).
- **Absorbency** (the weight of solution retained in a test sample) was expressed in grams of saline solution (0.9% solution of NaCl) that was absorbed in 1 cm^2 of the product sample submerged 1 cm below the surface of solution for 1 minute.
- **Adherence** was determined by adhesion of a sample (size 3 x 3 cm) to human skin after its wetting with saline solution.
- The **rate** and **uniformity of sample colouration in liquid** were visually evaluated in 0.1% $KMnO_4$ solution.
- **Shape stability** and **possibility of product (re)positioning** were visually evaluated when a product sample, after it had soaked in the test solution (either saline solution or 0.1% $KMnO_4$ solution), was pulled with a pair of tweezers onto a Petri dish, on which it was flattened, moved, lifted and placed again.
- **Bio-resorbability** was used to estimate the biological resorption of a material by monitoring the loss of the present sample in a buffered solution of enzymatically digested animal tissue (peptone). The unit of measurement was a whole day.

[1] A-Pharma s.r.o., contract research organisation, Study Report Ref. No. AP-SY-1901

Results

► **USP requirements** - **All measured values were within the limits of the US Pharmacopoeia for both products.**

Parameter	OKCEL® H-T	OKCEL® H-D	OKCEL® F	USP limits
	Measured values			
Loss on drying (1) (% mass)	7.7	8.0	8.1	≤ 15.0
Ash content (2) (% mass)	0.07	0.08	0.09	≤ 0.15
Nitrogen content (3) (% mass)	0.17	0.16	0.17	≤ 0.50
Formaldehyde content (4) (% mass)	0.02	0.02	0.02	≤ 0.50
Carboxyl group content (5) (% mass)	19.1	18.7	18.6	≥ 16.0 - ≤ 24.0
Identity (6)	Corresponds	Corresponds	Corresponds	Corresponds

Parameter	SURGICEL® Original	SURGICEL® NU-KNIT	SURGICEL® FIBRILLAR	USP limits
	Measured values			
Loss on drying (% mass)	7.4	7.8	6.2	≤ 15.0
Ash content (% mass)	0.05	0.04	0.05	≤ 0.15
Nitrogen content (% mass)	0.07	0.07	0.07	≤ 0.50
Formaldehyde content (% mass)	0.03	0.02	0.05	≤ 0.50
Carboxyl group content (% mass)	20.5	20.5	20.7	≥ 18.0 - ≤ 24.0
Identity	Corresponds	Corresponds	Corresponds	Corresponds

(1) API 11/1430/07
(2) API 11/1430/08
(3) API 11/1430/12
(4) API 11/1430/11
(5) API 11/1430/10
(6) API 11/1430/06

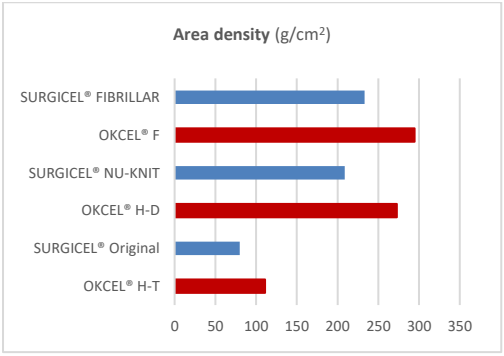
The sulfate ash content (showing the content of inorganic impurities) was found to be higher in *OKCEL®* which is probably due to a different production technology in which inorganic components predominate. These can be determined by this methodology, while organic components predominating in production of ORC are readily converted to gaseous components by annealing, and thus cannot be largely identified by this methodology.

The nitrogen content was also found to be higher for *OKCEL®* which can be attributed to the feedstock, natural cotton, in whose cell walls nitrogenous substances are commonly found.

The content of formaldehyde was found to be lower for all types of *OKCEL®*. Generally, it can be said that in spite of the adverse properties of formaldehyde, such as carcinogenicity and mutagenicity, it should not have a major impact on the patient's health if USP prescribed limit is maintained. In the body, formaldehyde is rapidly metabolized to formic acid, which is then incorporated into macromolecules and excreted in the urine, or oxidized to CO_2 excreted by the lungs.[2]

The carboxyl group content of *OKCEL®* was about 1.5% lower than that of *SURGICEL®*, which was expected with respect to USP requirements (with ORC values being between 18 and 24%, whereas for ONRC they are in the range from 16 to 24%). The degree of oxidation (carboxyl group content) is directly related to the resorption of oxycellulose in the body, where the rate of resorption increases with the degree of oxidation. Resorption occurs already at 10% content of carboxyl groups and, with the content of these groups ranging from 12-24%, the material is absorbed within 1 month after implantation.[3] In view of the nonlinear relation between % COOH and the resorption of oxycellulose in the body, the carboxyl content values for both ONRC and ORC can be considered comparable in terms of resorbability. If both ONRC and ORC meet USP requirements, resorption will be more influenced by the amount of oxycellulose used, the degree of blood saturation and the condition of the tissue bed.

► **Area density** - All ***OKCEL®* product types had a higher area density** against comparable *SURGICEL®* products. This is **one of the factors that support the fluid absorbency resulting in better haemostatic effect**. Looking at this parameter in relation to absorbency, it is evident that *OKCEL®* exceeded *SURGICEL®* in the absorbency parameter to a greater extent than the difference in area density, and therefore that absorbency of *OKCEL®* was positively affected also by other factors.



► **pH –** In general, **lower pH values have a positive effect on haemostasis and antimicrobial properties**. In this study, *OKCEL®* achieved a lower pH value, when compared to *SURGICEL®* products, in case of *OKCEL® F* (3.0) versus *SURGICEL® FIBRILLAR* (3.2). *OKCEL® H-D* and *SURGICEL® NU-KNIT* achieved identical results (3.3), also *OKCEL® H-T* and *SURGICEL® Original* were at a comparable level (3.2). However, with regard to the possibility of changing the pH value due to the hydrolysis of oxycellulose during its shelf life, the values cannot be unambiguously considered "side by side".

In this context, it is worth mentioning that antimicrobial activity of *OKCEL®* (bacteriostatic or bactericidal) has been demonstrated against 36 strains, including antibiotic-resistant bacteria (MRSA, MRSE, VRE, PRSP)[4].

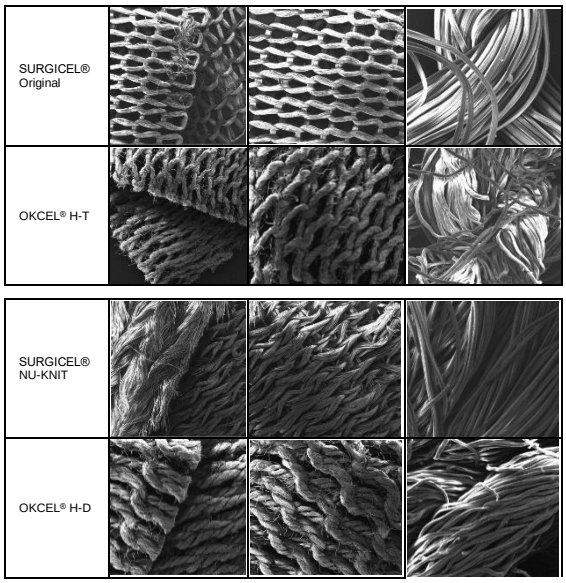
► **Structure** - The overall appearance (column density, rows and bonding) of both types of knitted fabric (*SURGICEL® Original x OKCEL® H-T* and *SURGICEL® NU-KNIT x OKCEL® H-D*) was very similar. However, at a magnification of 50x, there was already a considerable difference in the hairiness of the yarn used. *OKCEL®*

2 Kazmarová, H., Velická, H. Jsou problémy s formaldehydem minulostí? Aktuální pohled na zdravotní účinky [Internet].[Czech Republic]: Státní zdravotní ústav; 2010. 24p. Available at: http://www.szu.cz/uploads/documents/chzp/ovzdusi/konz_dny_a_seminare/2010/08_formaldehyd_2010.pdf

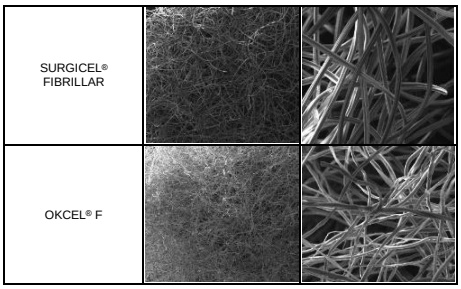
3 Domb, A.J., Kost, J., Wiseman, D. M. Handbook of Biodegradable polymers, CRS Press: 1998. 544 p.

4 Data on file. Ing. Iveta Brožková, Ph. D. at al. Department of Biological and Biochemical Sciences at the Faculty of Chemical Technology, University of Pardubice. Final report on testing the antimicrobial activity of the product OKCEL. 18640 - 034.

samples were significantly hairier than *SURGICEL®*. At a magnification of 500x, a larger number of individual fibre ends could also be seen in *OKCEL®* products which is indicative of the presence of shorter fibres. Furthermore, there was visible a difference in the thickness of individual fibres, with the fibres of *OKCEL®* products reaching a maximum thickness of 17 µm, while the fibres of *SURGICEL®* products ranged up to 23.5 µm.

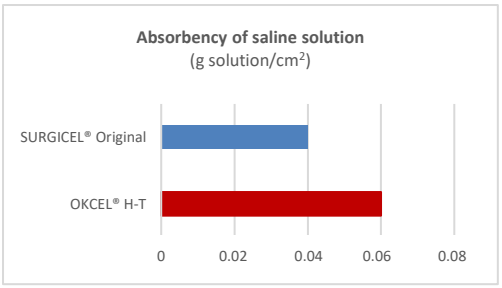


Similar results were also evident in samples of nonwoven fabric (*SURGICEL® FIBRILLAR x OKCEL® F*).



The above documented hairiness, a greater surface area and inter-fibrous space and a larger number of frayed fibres are likely to result in increased fluid absorbency of ONRC products[5].

► **Absorbency** - ***OKCEL®*** **exhibited higher fluid absorbency** compared to *SURGICEL®*, which can be largely attributed to the increased area density of *OKCEL®* compared to *SURGICEL®* and also to the properties identified by the electron microscope (larger surface area along with frayed fibres). **The higher absorbency of *OKCEL®* products will have a positive clinical impact as it will support the rate of haemostasis.**

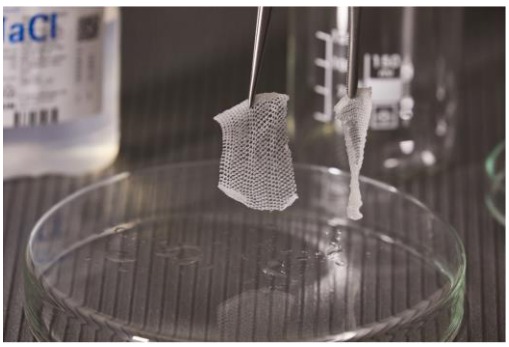


► **Adherence** – The adherence to human skin was satisfactory for all products, the examined samples were flexible, adhered to the skin, the edges did not twist, samples did not fall off the skin and were fully soaked throughout the area.

► **Rate and uniformity of sample colouration in liquid** – Both types of oxidized cellulose absorbed the solution immediately and evenly.

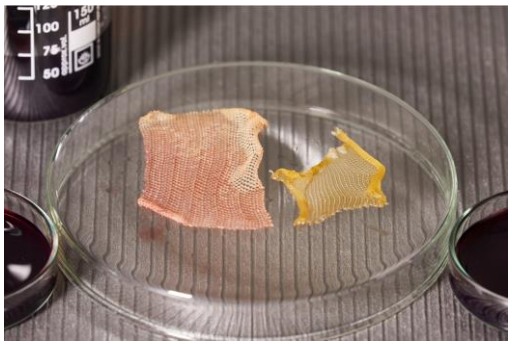
► **Shape stability and possibility of product (re)positioning** - After *SURGICEL®* samples were soaked in the test solution and were pulled with a pair of tweezers onto a Petri dish, it was relatively difficult to manipulate them. Samples very soon (virtually immediately) failed to retain their original shape as well as size, when they shrunk quite significantly, and were generally difficult to straighten. In contrast, *OKCEL®* samples were handled without any problems, they were compact, and retained their original shape and size.

To sum up, these tests demonstrated comparable adherence and colouration of both kinds of haemostat with better shape stability and the possibility of repositioning of *OKCEL®* versus *SURGICEL®*. *OKCEL®* retains its' original structure after fluid saturation and can be thus easily manipulated at the bleeding site without any signs of disengaging which **provides benefits for surgeons as it greatly simplifies material handling during surgical procedures.**



Shape stability after the adherence test *– knitted fabric (OKCEL® H-T on the left side, SURGICEL® Original on the right side)*

5 K. M. Lewis, DVM, D. Spazierer, PhD, M. D. Urban, HT (ASCP), L. Lin, PhD, H. Redl, PhD, A. Goppelt, PhD. Comparison of regenerated and non-regenerated oxidized cellulose hemostatic agents. [on-line]. Eur Surg. 2013; 45(4): 213–220.

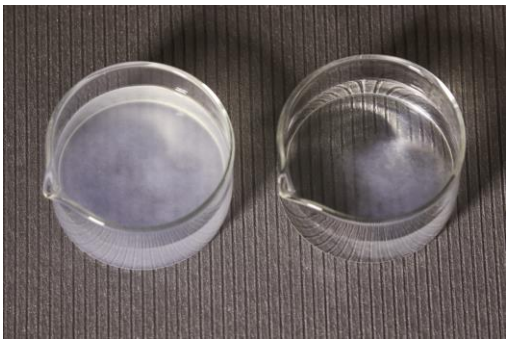


Shape stability after the colouration test *– knitted fabric (OKCEL® H-T on the left side, SURGICEL® Original on the right side)*

► **Bio-resorbability** - This parameter is particularly important in clinical practice since a good resorption in the body is a prerequisite for a smooth and uncomplicated postoperative course. To estimate the resorption of both products in the body, a simple bio-resorbability test simulating contact with body fluids during surgery was performed. The test was carried out for *OKCEL® H-T* against *SURGICEL® Original*.

This in-vitro bio-resorbability simulation test demonstrated better disintegration of *OKCEL®* versus *SURGICEL®.* The structure of knitted fabric *OKCEL® H-T* has loosened into individual fibres after 7 days of immersion in peptone **while *SURGICEL® Original* was in the form of a relatively compact gel clot**, which could be almost completely removed from the peptone solution using tweezers. **The *SURGICEL® Original* sample loosened into single fibres under the same conditions as *OKCEL® H-T* after 12 days.** The final phase of loosening the structure into individual fibres was not visually identical. While individual *OKCEL®* fibres were dispersed homogeneously throughout the solution volume, the *SURGICEL®* fibres still formed a clot in an otherwise clear solution, but could no longer be removed with tweezers.

In vivo, this could cause the formation of encapsulated fluid collection which is very often misdiagnosed during post-surgical imaging follow-up as it tends to mimic abnormalities such as abscesses, tumors or retained foreign bodies (this issue of granuloma mimicking tumor formation after the use of *SURGICEL®* has been addressed in lots of previous observations*).*[6,7,8]



Bio-resorbability after 7 days *– knitted fabric (OKCEL® H-T on the left side, SURGICEL ® Original on the right side)*

Bio-resorbability after 7 days *– knitted fabric (OKCEL® H-T on the left side, SURGICEL® Original on the right side)*

Bio-resorbability after 7 days *– knitted fabric (OKCEL® H-T on the left side, SURGICEL® Original on the right side)*

6 Cormio, L. et al. Surgicel® granuloma mimicking ovarian cancer: A case report. Oncol Lett. 2016 Aug; 12(2): 1083–1084. doi: 10.3892/ol.2016.4689

7 Gao, H.W. et al. Oxidized cellulose (Surgicel) granuloma mimicking a primary ovarian tumor. Int J Gynecol Pathol. 2002 Oct;21(4):422-3.

8 Wang, H. et al. Surgicel® (oxidized regenerated cellulose) granuloma mimicking local recurrent gastrointestinal stromal tumor: A case report. Oncol Lett. 2013 May; 5(5): 1497–1500. doi: 10.3892/ol.2013.1218

Conclusion

Based on this comparative study, it can be said that both tested haemostats are comparable in USP and basic physicochemical parameters.

With regard to the area density, one of the factors contributing to increased absorbency and a better haemostatic effect, *OKCEL®* products achieved higher values compared to *SURGICEL®* products.

Looking at the pH parameter, whose lower values have a positive effect on haemostasis and antimicrobial properties, *OKCEL®* non-woven product (*OKCEL® F*) showed lower pH value than comparable *SURGICEL®* product (*SURGICEL® FIBRILLAR*). In the case of knitted fabrics (*OKCEL® H-T, OKCEL® H-D* and *SURGICEL® Original*, *SURGICEL® NU-KNIT*), differences in pH were not considered significant.

Both products were comparable in adherence and colouration in liquid but sticking to instruments (tweezers) was only observed for *SURGICEL®* products. Easier handling with *OKCEL®* was in general supported by its shape stability, which allows the haemostatic material to be further manipulated and corrected even after placement at the bleeding site.

***OKCEL®* exhibited higher absorbency, which is attributable to the higher area density and the larger surface area along with frayed fibres. The higher absorbency of *OKCEL®* products has a positive clinical impact as it supports the rate of haemostasis.**

The in vitro bio-resorbability simulation test, comparing the knitted fabrics *OKCEL® H-T* versus *SURGICEL® Original*, demonstrated better disintegration of *OKCEL®* which had loosened homogeneously into individual fibres in a shorter time, without creating a compact clot of material as was the case with *SURGICEL®*. As it has been reported in some literature, this retained material can be seen to cause in vivo a foreign-body granuloma imitating different pathological conditions which complicates post-surgical imaging.

In summary, *OKCEL® is a* haemostatic material that is characterized by such utility properties as easy handling, possibility of (re)positioning and shape stability. Its other qualities are fast bioresorbability/biodegradability and high haemostatic capacity which is supported with its high absorbency and large surface area.

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