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Microwave-assisted synthesis and characterisation of biomaterials based on chitosan

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The Team

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Chitosan

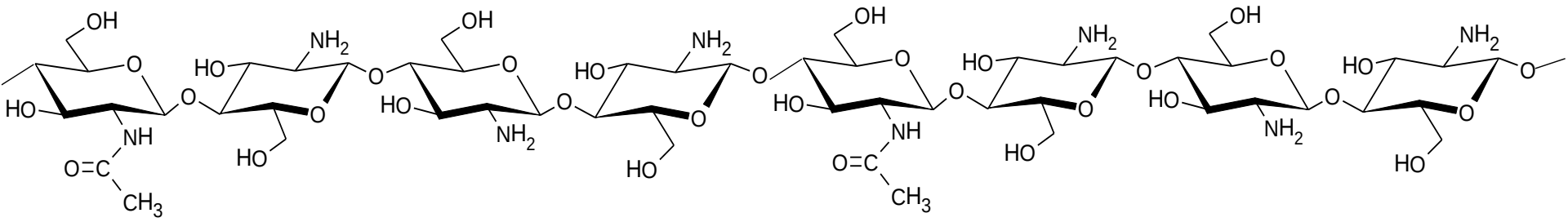


Fig. 1. Chemical structure of chitosan

- **Antibacterial**
- **Biodegradable**
- **Biocompatible**
- **Antioxidant**
- **Hemostatic**

Obtained through chitin deacetylation

Synthesis

chitosan

amino acids

glycol

Series J – mesenchymal stem cells culture

Series K – keratinocytes culture

Microwave radiation

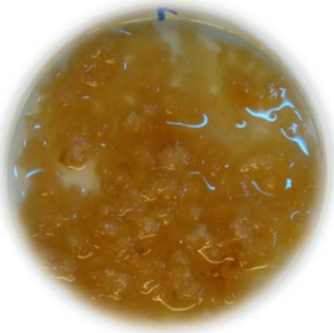
Synthesis

Reaction mixture

Reaction vessel

Prolabo Synthwave 402 microwave reactor

Purification



Obtained hydrogel

Synthesis parameters

Tab. 1. Synthesis parameters for Series J

Sample	Asp : Glu : chitosan [g]	Glycol [ml]	Time [min]	Power [W]	Temp. [°C]
J1	0,1 : 0,1 : 1,0	6	30	300	150
J2	0,1 : 0,1 : 1,0	6	30	500	180
J3	0,2 : 0,1 : 1,0	6	30	300	150
J4	0,2 : 0,1 : 1,0	6	30	500	180
J5	0,1 : 0,2 : 1,0	6	30	300	150
J6	0,1 : 0,2 : 1,0	6	30	500	180
J7	0,2 : 0,2 : 1,0	6	30	300	150
J8	0,2 : 0,2 : 1,0	6	30	500	180
J9	0,3 : 0,3 : 1,0	6	30	300	150
J10	0,3 : 0,3 : 1,0	6	30	500	180

Steam sterilisation

EO sterilisation

Synthesis parameters

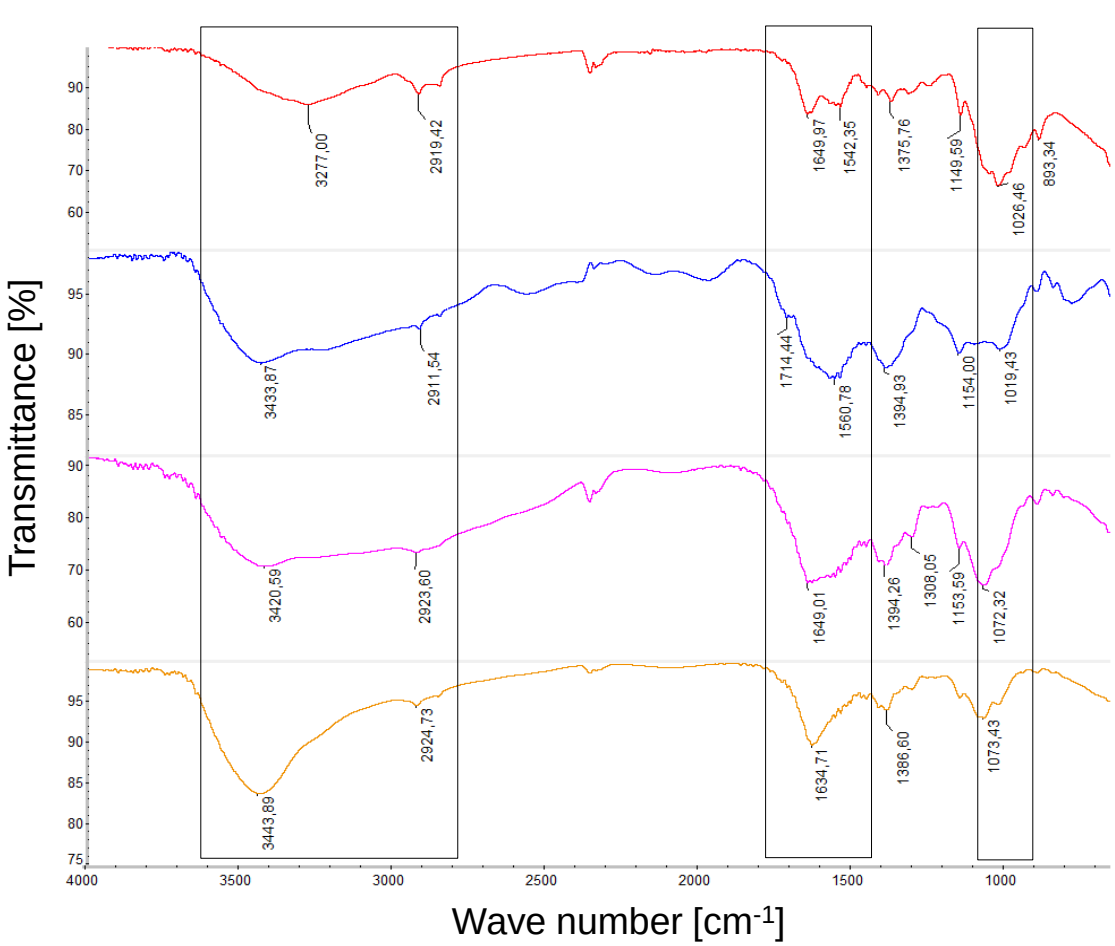
Tab. 2. Synthesis parameters for Series K

Sample	Asp : Glu : chitosan [g]	Glycol [ml]	Time [min]	Power [W]	Temp. [°C]
K58	0,10 : 0,20 : 1,00	10	25	300	150
K59	0,10 : 0,20 : 1,00	10	25	500	180
K60	0,20 : 0,10 : 1,00	10	25	300	150
K61	0,20 : 0,10 : 1,00	10	25	500	180
K62	0,20 : 0,20 : 1,00	10	25	300	150
K63	0,20 : 0,20 : 1,00	10	25	500	180
K64	0,25 : 0,20 : 1,00	10	25	300	150
K65	0,25 : 0,20 : 1,00	10	25	500	180
K66	0,25 : 0,25 : 1,00	10	25	300	150
K67	0,25 : 0,25 : 1,00	10	25	500	180

Gamma radiation sterilisation (25 kGy)

Gamma radiation sterilisation (35 kGy)

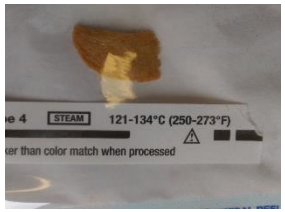
FTIR analysis - Series J



Chitosan

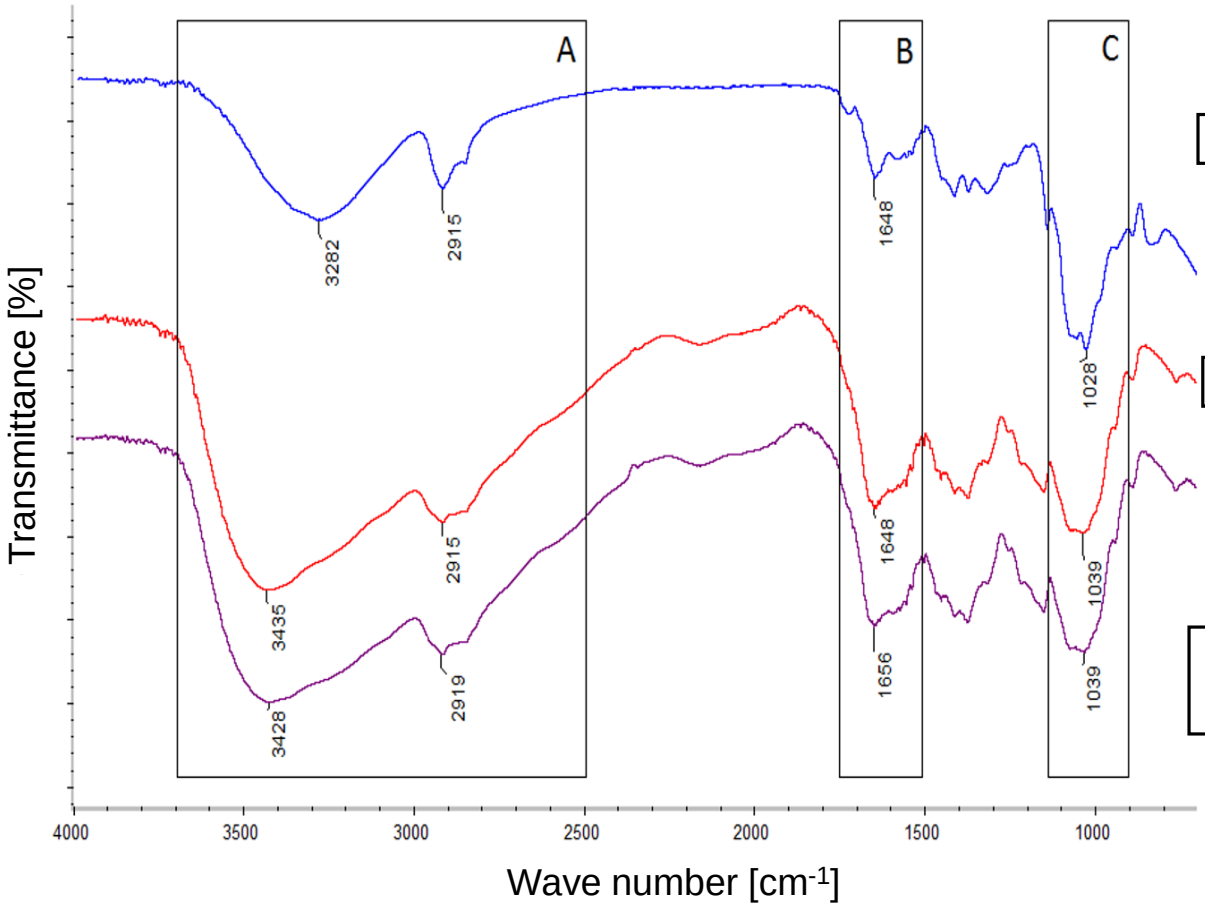
Scaffold

Thermal sterilisation



Chemical sterilisation

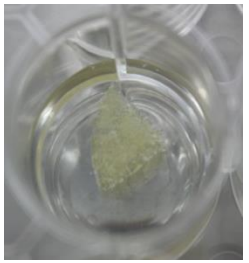
FTIR analysis - Series K



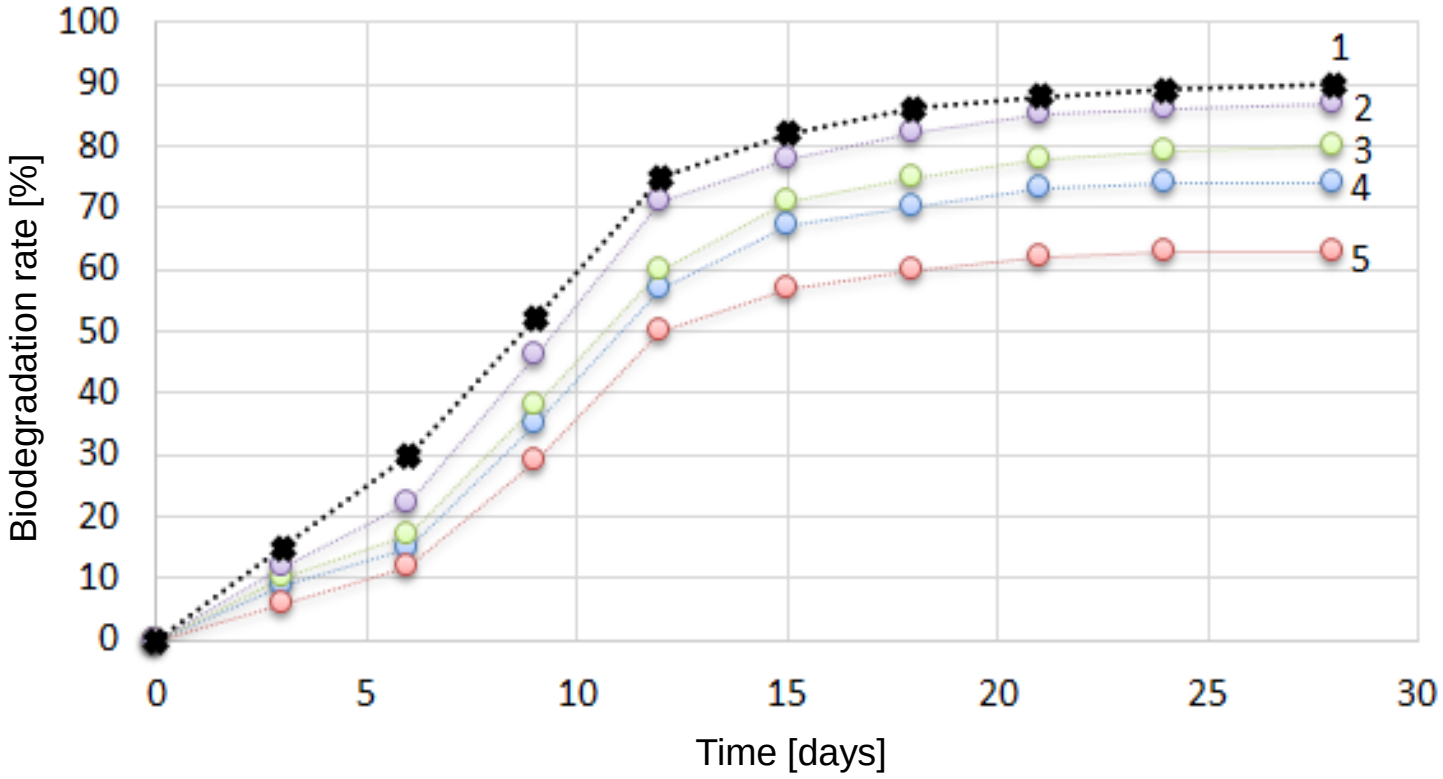
Chitosan

Scaffold

Radiation sterilisation

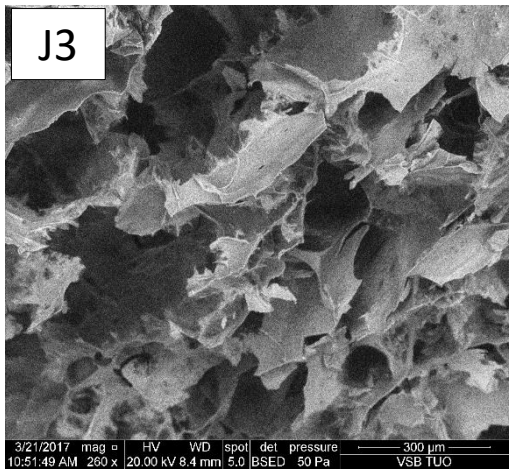


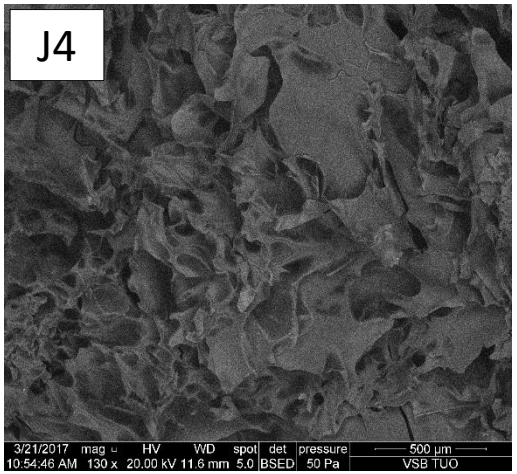
Biodegradation – Series J

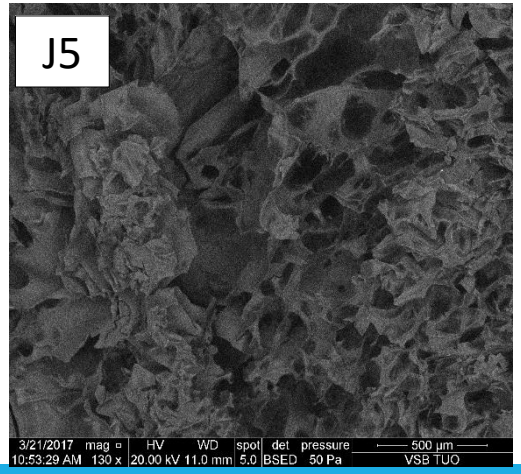


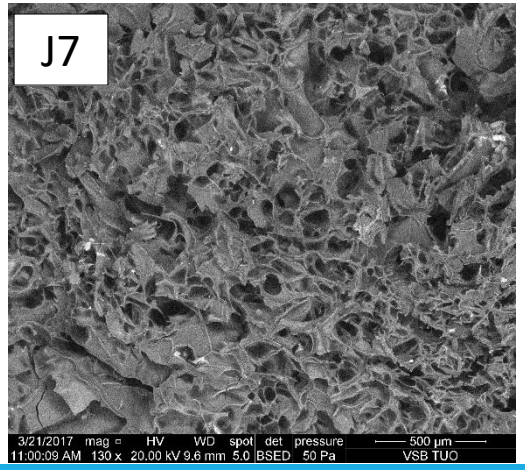
1 – sodium acetate
2 – J3
3 – J4
4 – J5
5 – J7

SEM analysis – Series J









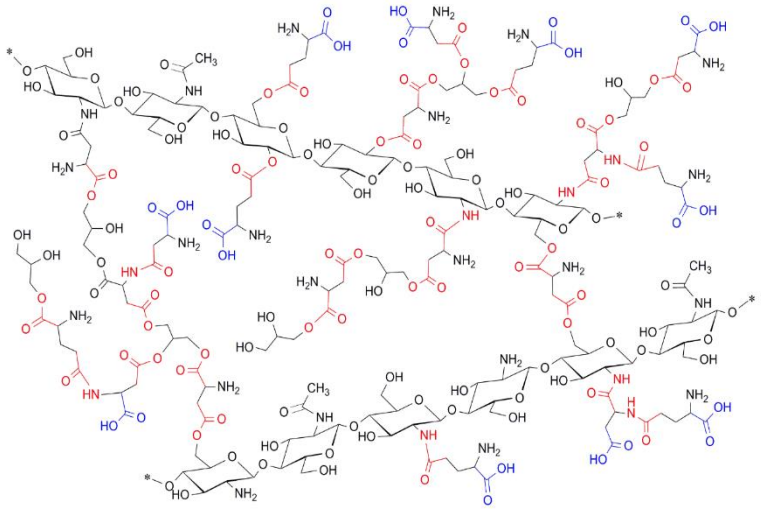


Fig. 2. Proposed chemical structure of crosslinked chitosan

Antibacterial properties - Series J

Tab. 3. Growth of bacteria on prepared scaffolds

Sample	AmnioGrow	DMEM	KSFM
J3	-	-	-
J4	-	-	-
J5	-	-	-
J7	-	-	-

Both scaffolds before and after sterilization in all tested media were not colonized by bacteria and fungi.

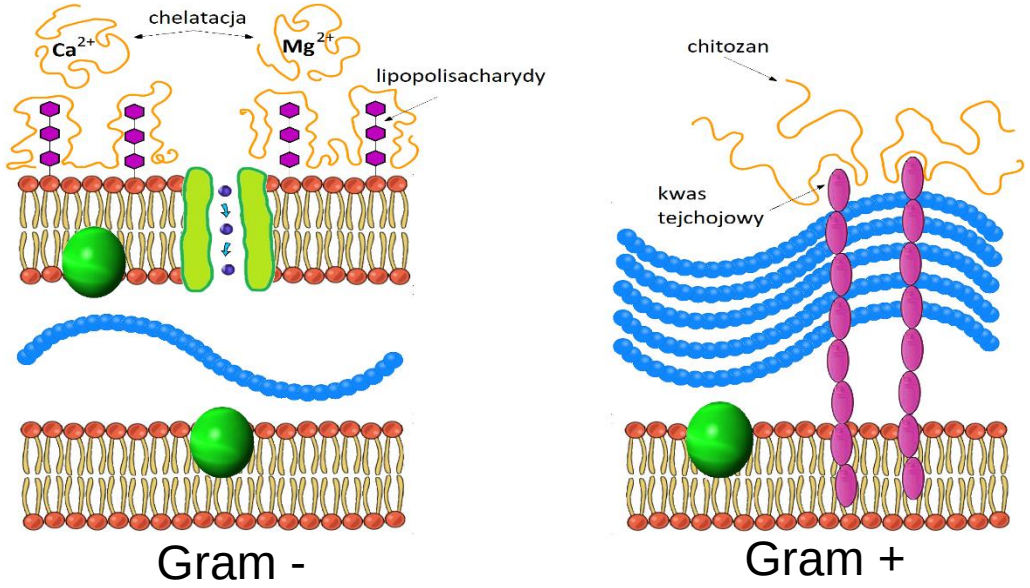
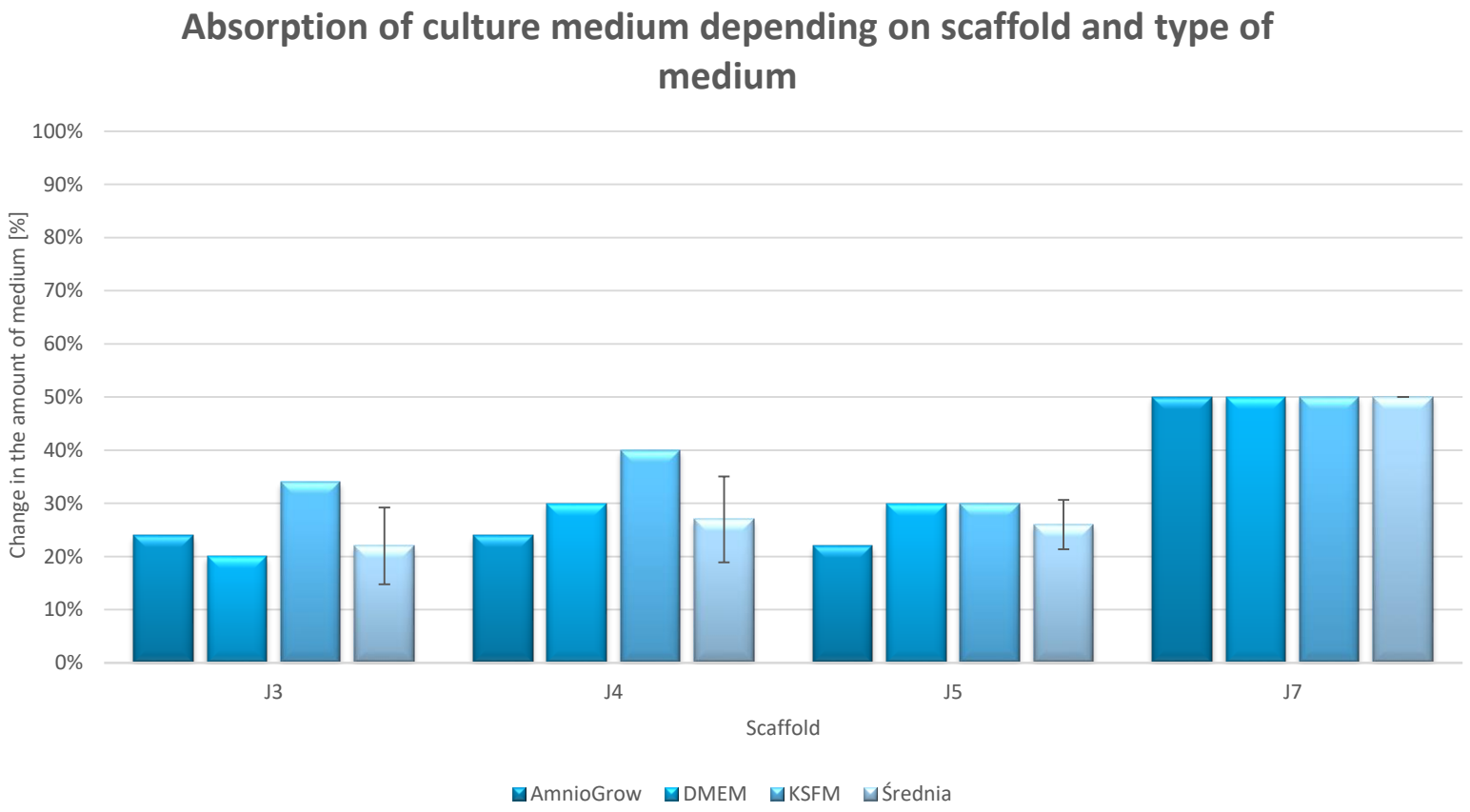


Fig. 3. Mechanism of antibacterial activity of chitosan

Swelling - Series J



Stability in culture media - Series J

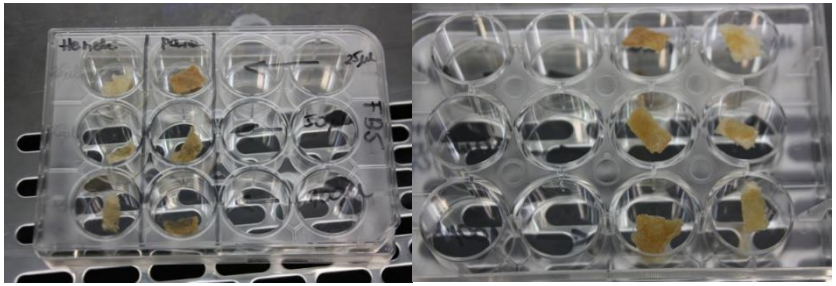


Fig. 4. Attachment to the bottom of the plate (J7)

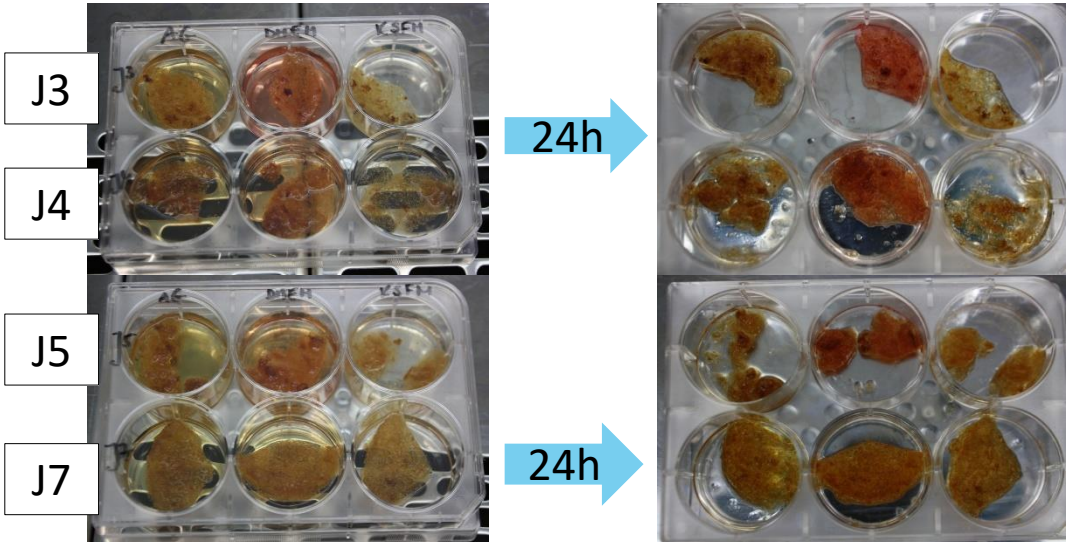
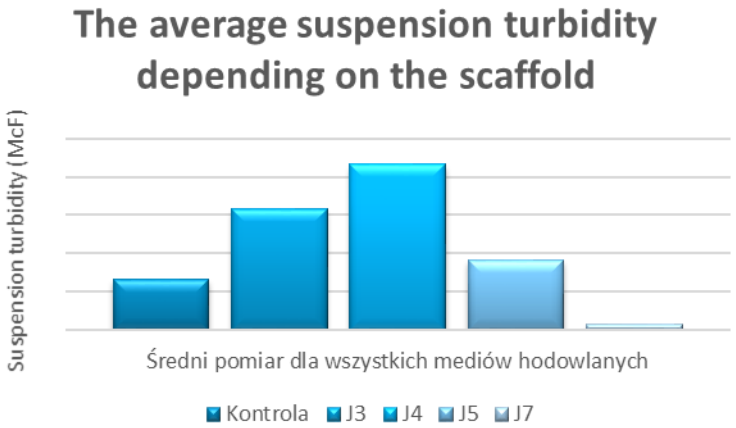
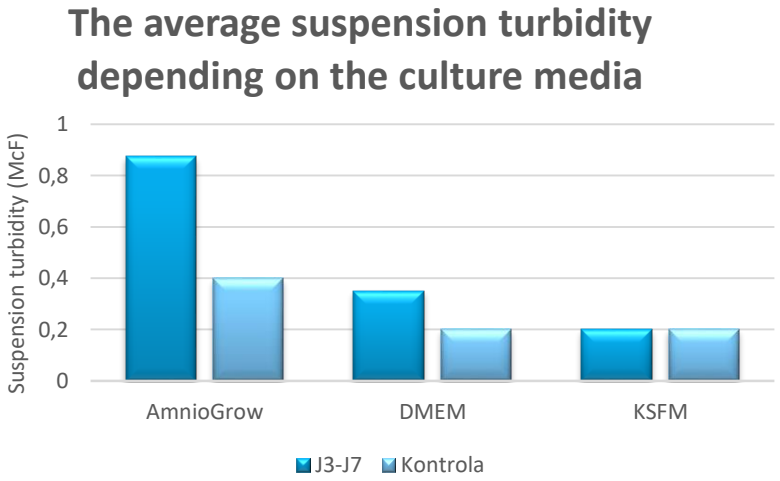
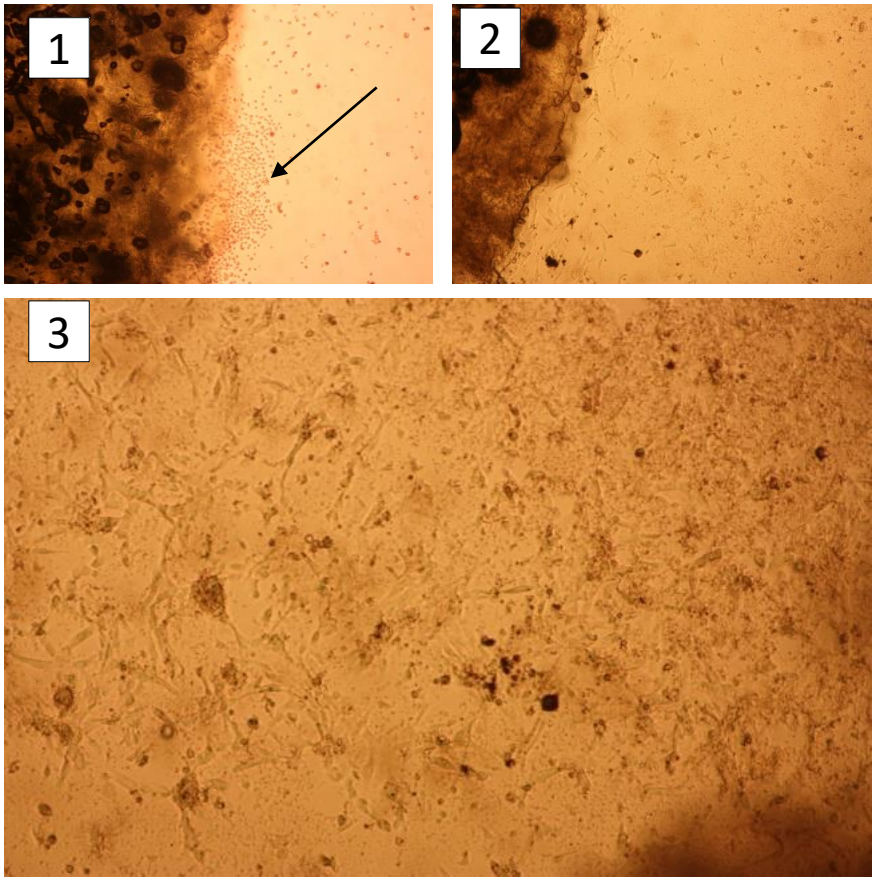


Fig. 5. Swelling in culture media





Population doubling - Scaffold J7



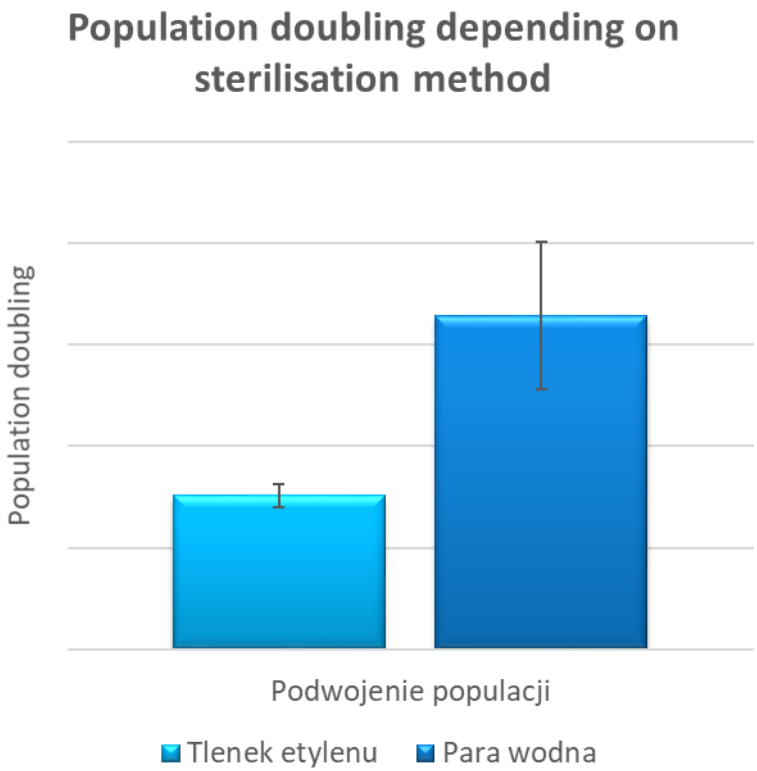
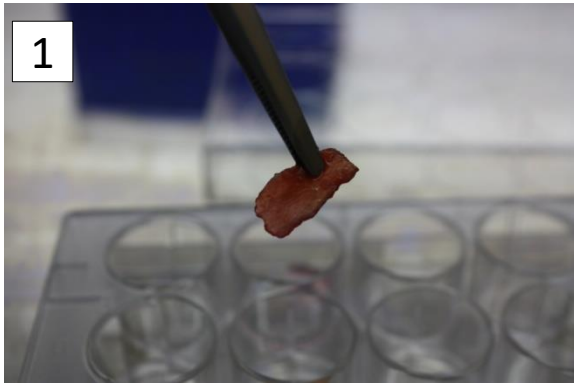
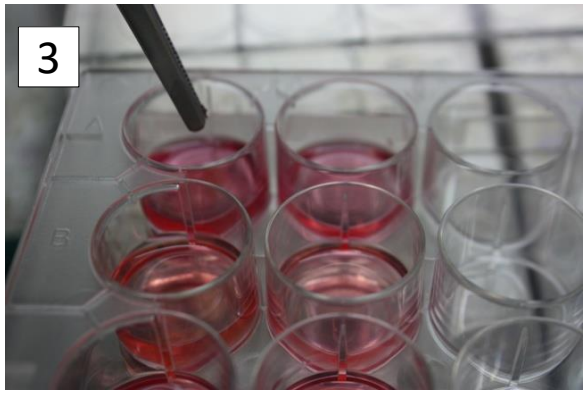
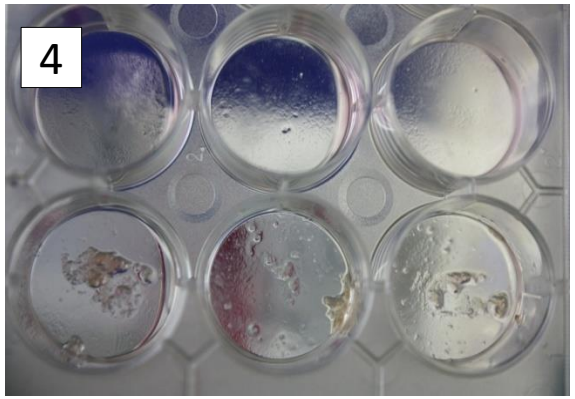


Fig. 6. 1 – Sown cells, 2 – No contact inhibition, cell coating, 3 - Proliferation

Scaffold stability - J7







1. Manipulation with tweezers doesn't affect the integrity
2. Scaffold have retained its form after the transfer
3. Disintegration of scaffold sterilised with ethylene oxide
4. Fragments of scaffold sterilised with EO permanently attached to the plate

Fig. 7. Stability of the scaffold after cell culture

Cells settlement

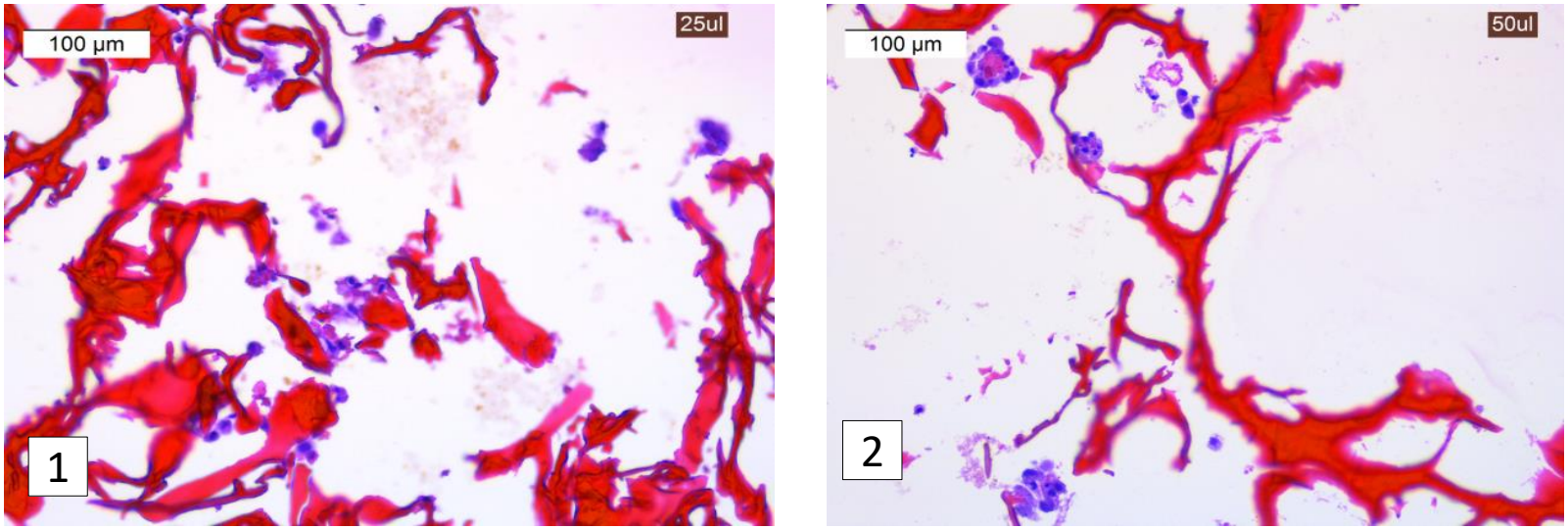
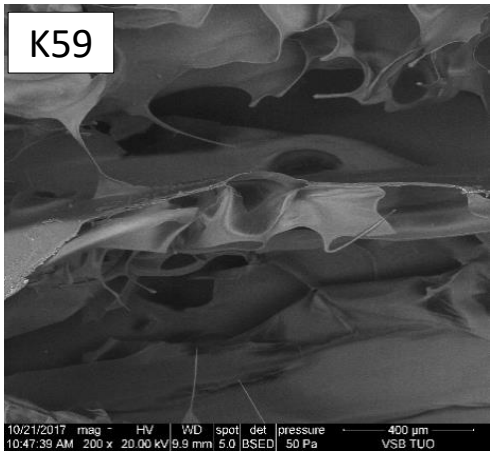
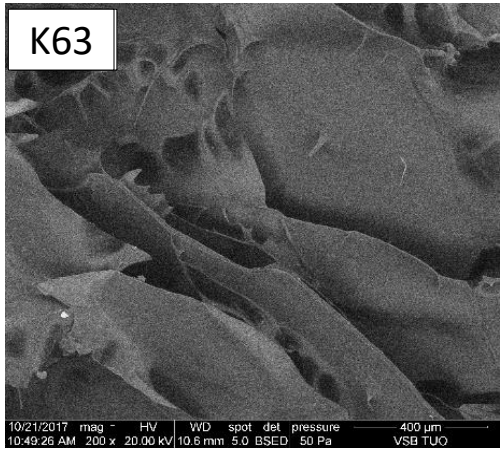


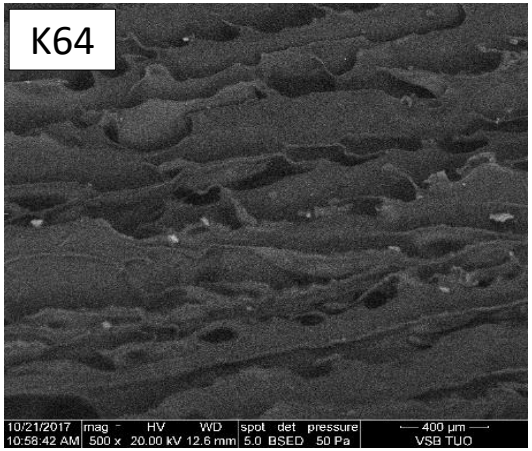
Fig. 8. Histopathological analysis showing the presence of cells in chitosan scaffold, sterilized both with steam (1) and with ethylene oxide (2)

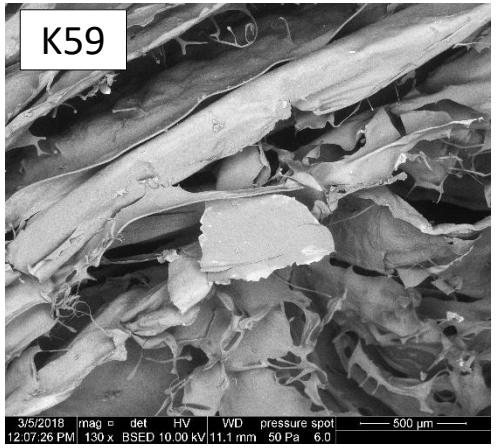
Visible blue-violet stained cells and matrix fibres stained in red

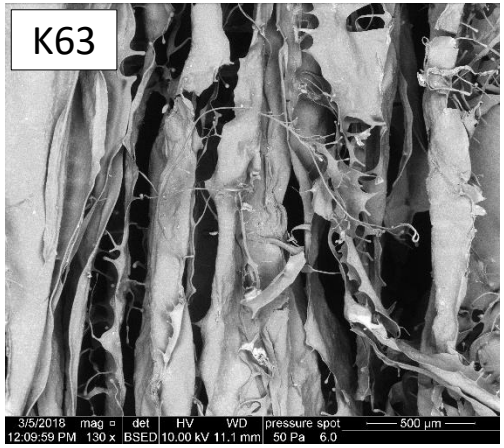
SEM analysis – Series K

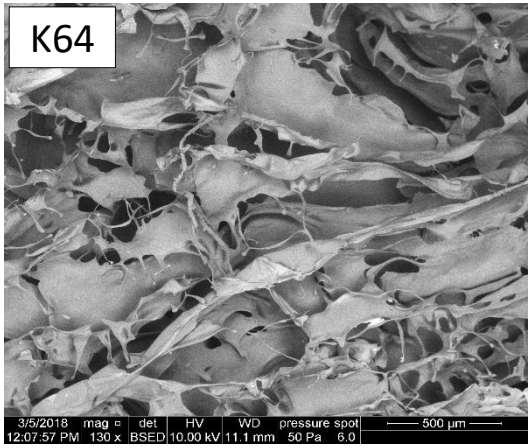










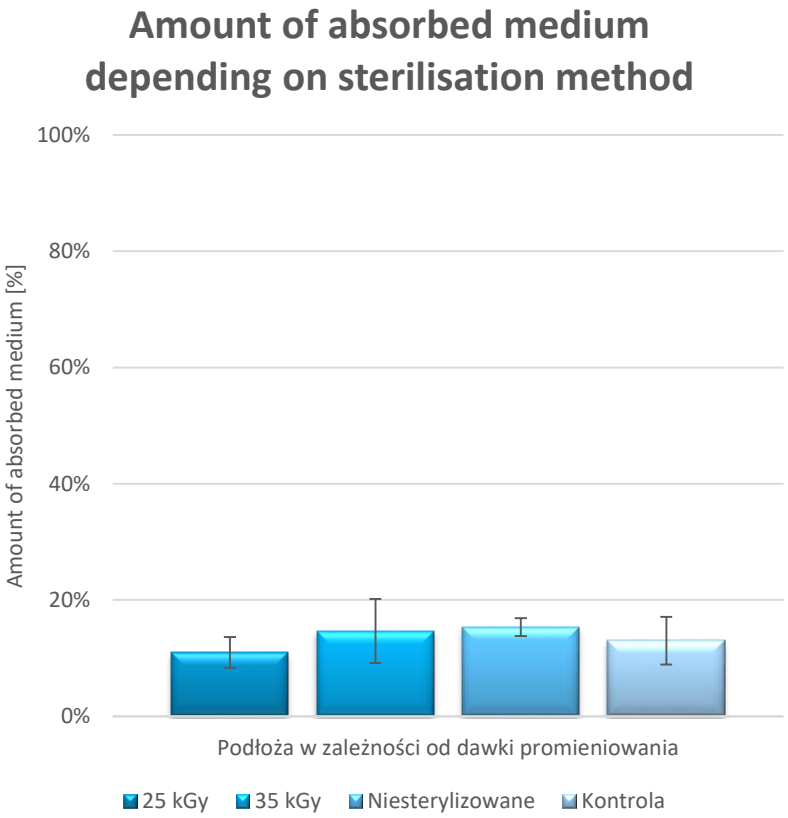


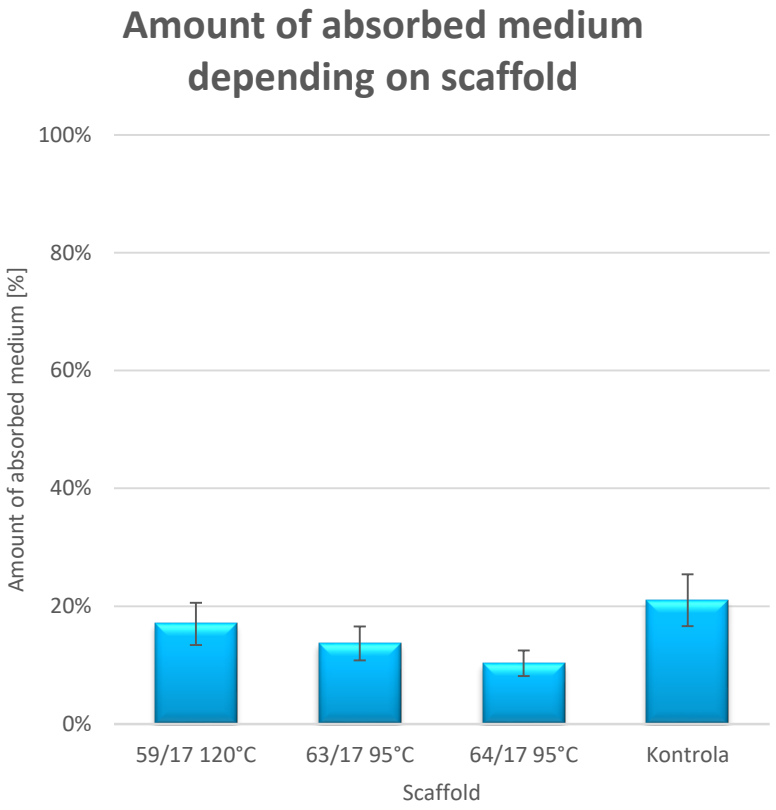
Scaffold sterility - Series K

Tab. 4. Growth of bacteria on prepared scaffolds

	K63/ 95°C	K64/ 95°C	K59/ 120°C
Before sterilisation	S. epidermidis MSSE	-	S. epidermidis MRSE
Sterility in culture with antibiotics before radiation	-	-	-
Sterility after irradiation (25 kGy)	-	-	-
Sterility after irradiation (35 kGy)	-	-	-
Sterility in culture without antibiotics after irradiation (25 kGy)	-	-	-
Sterility in culture without antibiotics after irradiation (35 kGy)	-	-	-

Swelling - Series K





Cells adherence - Series K

Tab. 5. Percentage expressed number of cells that did not adhere to the bottom of the culture vessel depending on the dose of sterilization radiation and the type of medium

	K59/ 120°C	K63/ 95°C	K64/ 95°C	Control
Unsterile	3,7%	2,9%	5,7%	6,5 %
Sterilised with 25kGy	2,6%	13,6%	6,5%	
Sterilised with 35kGy	14,3%	5,5%	4,1%	
Average	6,8%	7,3%	5,4%	

Cells adherence - Series K

Cells adherence; sterilisation with 25 kGy after 96h

Cells adherence; sterilisation with 35 kGy after 96h

Degree of enzymatic detachment

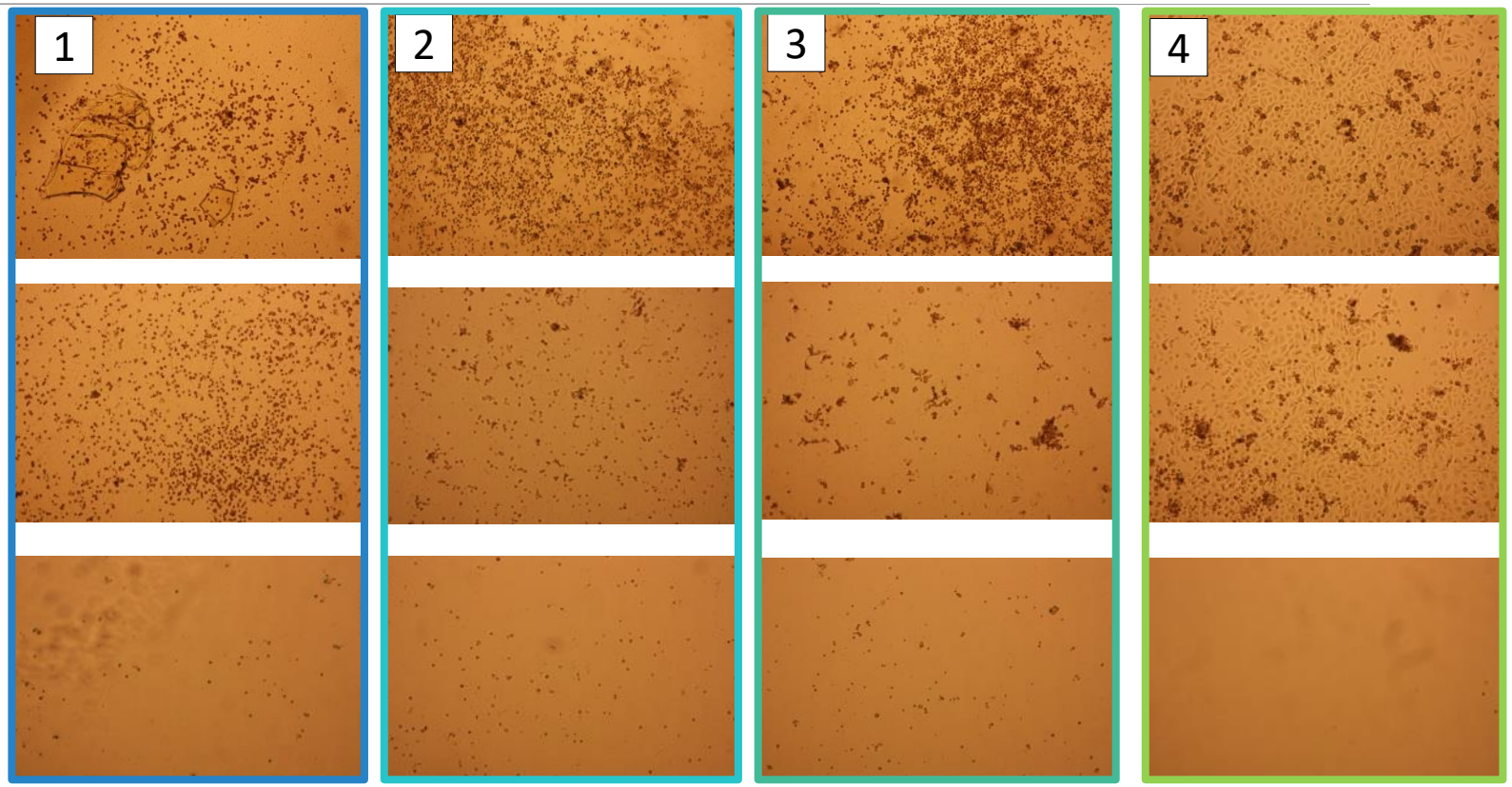
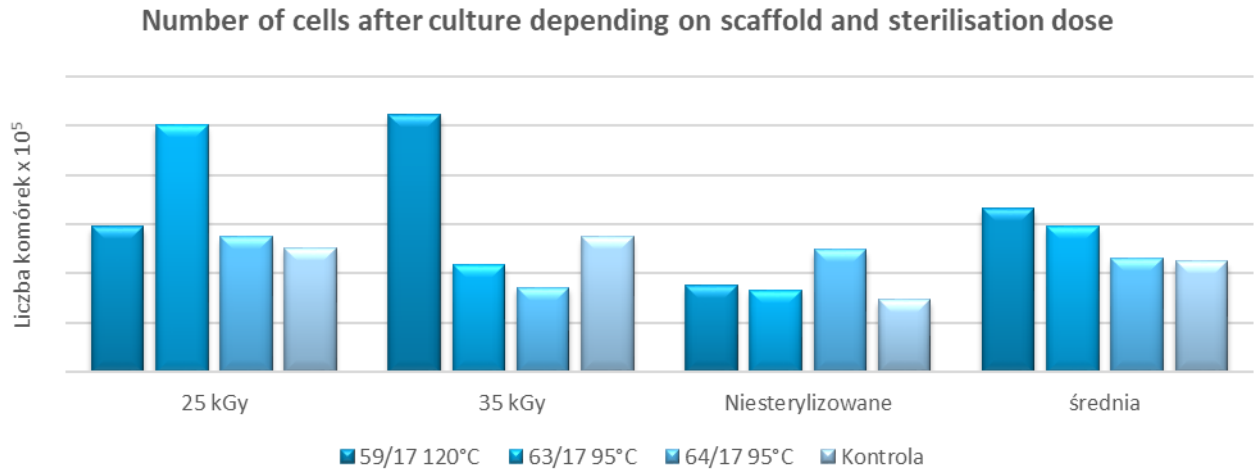
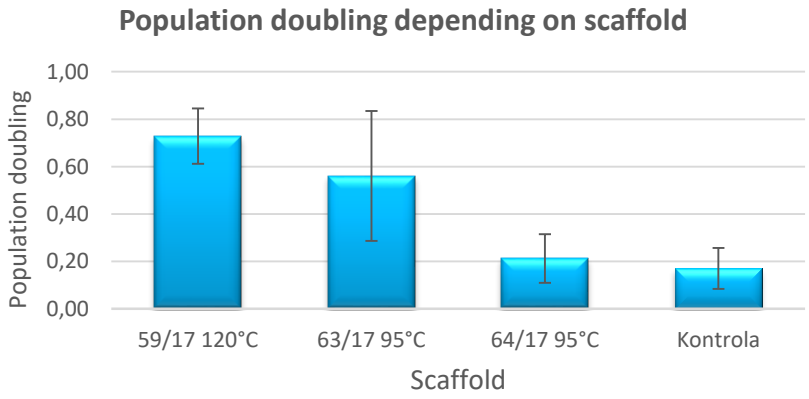
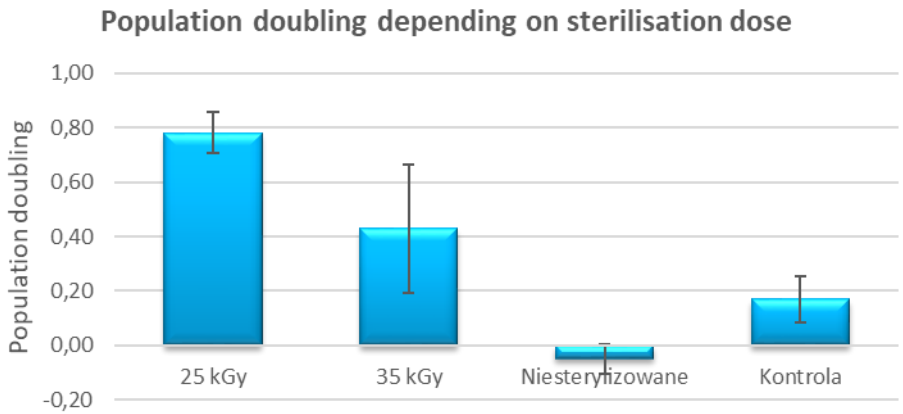


Fig. 9. 1 - Sample K59, 2 - Sample K63 , 3 - Sample K64, 4 - Control

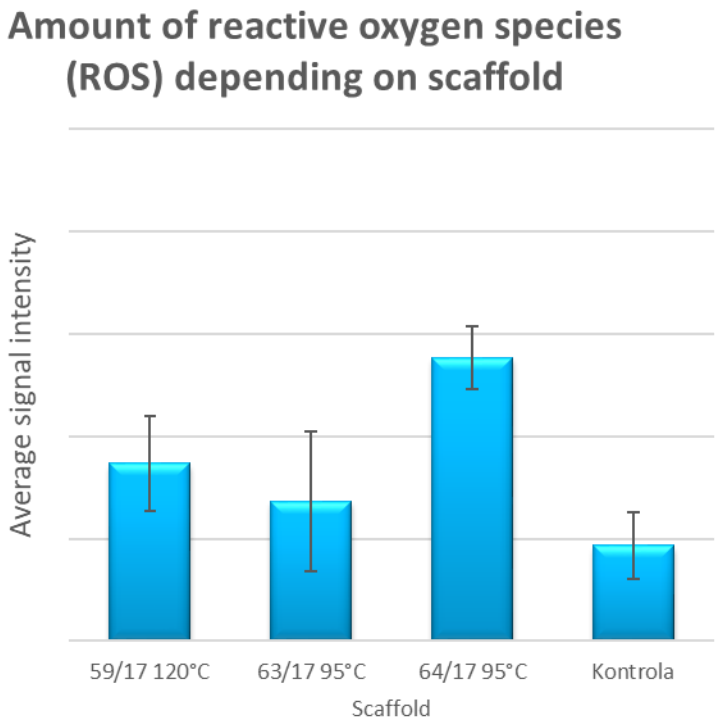
Population doubling – Series K

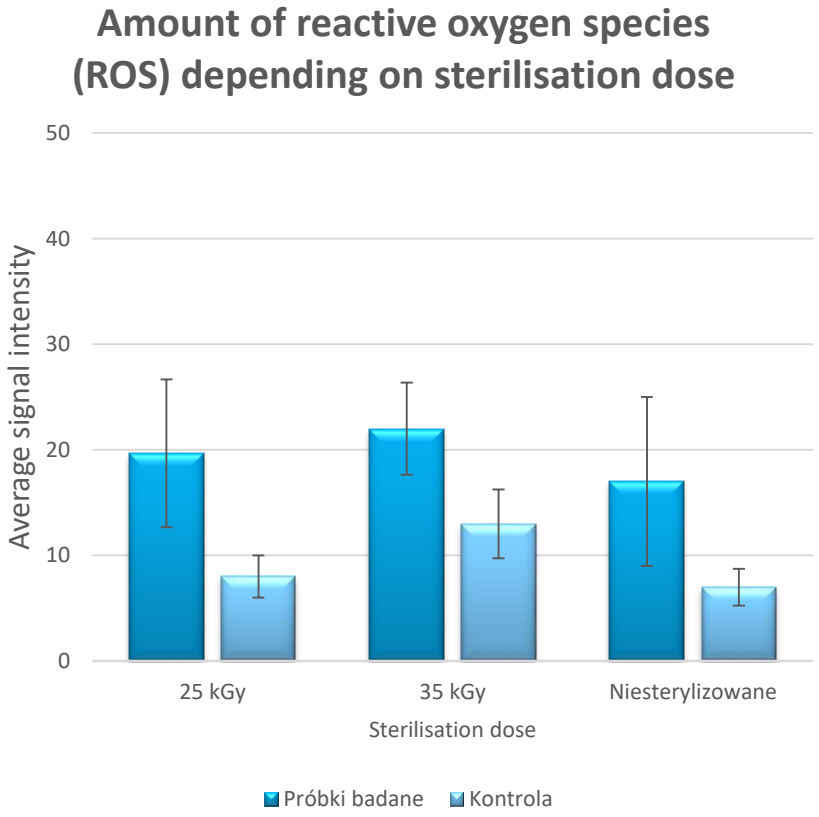




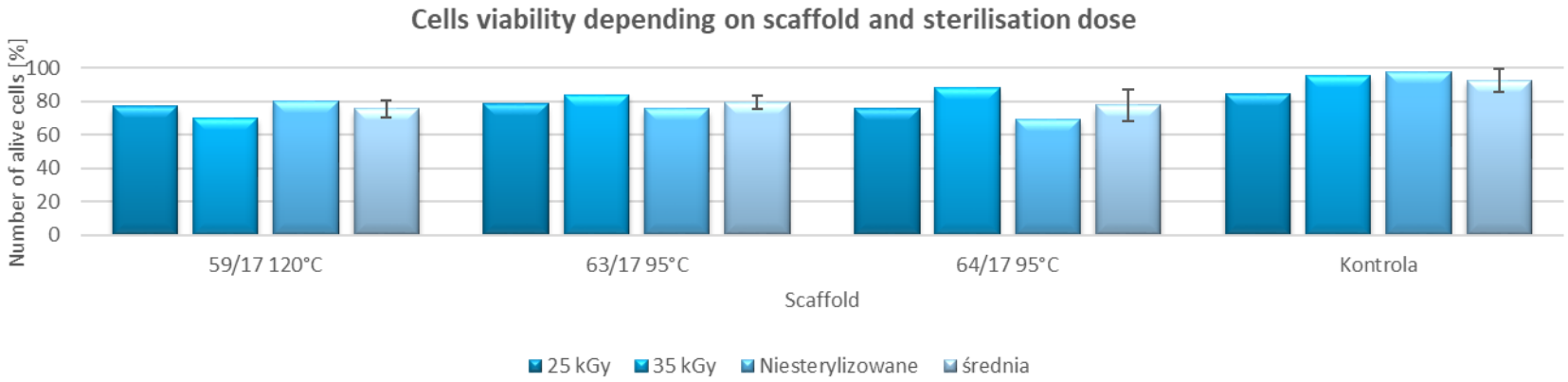


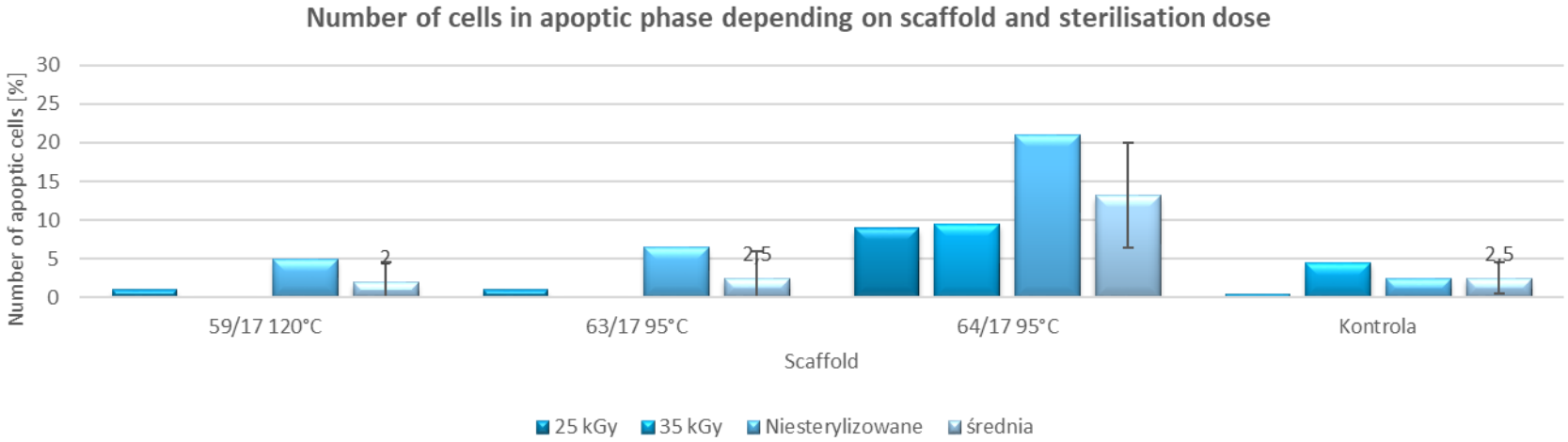
Reactive Oxygen Species - Series K





Cells viability - Series K





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