



Aims of the study:

- To synthesise bacterial cellulose (BC) hydrogel and silver nanoparticles (Ag-NP)
- To load Ag-NP in purified BC hydrogel
- To determine antimicrobial activity of Ag-NP loaded BC against gram positive (+ve) and gram negative (-ve) bacteria for its potential application as an antimicrobial hydrogel wound dressing

Why Silver (Ag) and Why Ag-NP?

- Silver (Ag)** is a broad spectrum antimicrobial agent (fig. 1) which:
 - > Inhibits bacterial enzymes
 - > Interferes with electron transport
 - > Binds to DNA
- Ag** displays antimicrobial activity [4, 5] against:
 - > Yeast
 - > Fungi
 - > Even antibiotic resistant bacteria including MRSA
- Ag-NP** act in 3 different ways [6] (fig. 2):
 - > Attach to the surface of bacterial cell membrane, disturbs permeability
 - > Penetrate inside the bacterial cell and cause damage
 - > Release Ag⁺ and have added bactericidal effect

Why BC hydrogel?

- BC is:
 - > Biosynthetic hydrogel
 - > Biocompatible, non-pyrogenic [1, 2]
 - > Chemically pure [3]
 - > Transparent [2]
- Due to its properties BC have generated commercial applications in the wound management sector including XCell[®], Bioprocess[®], Gengiflex[®] [2].

For your attention: *G. xylinus* is a rod-shaped Gram -ve bacterium capable of producing cellulose pellicles which upon purification can be used as moist wound dressings.

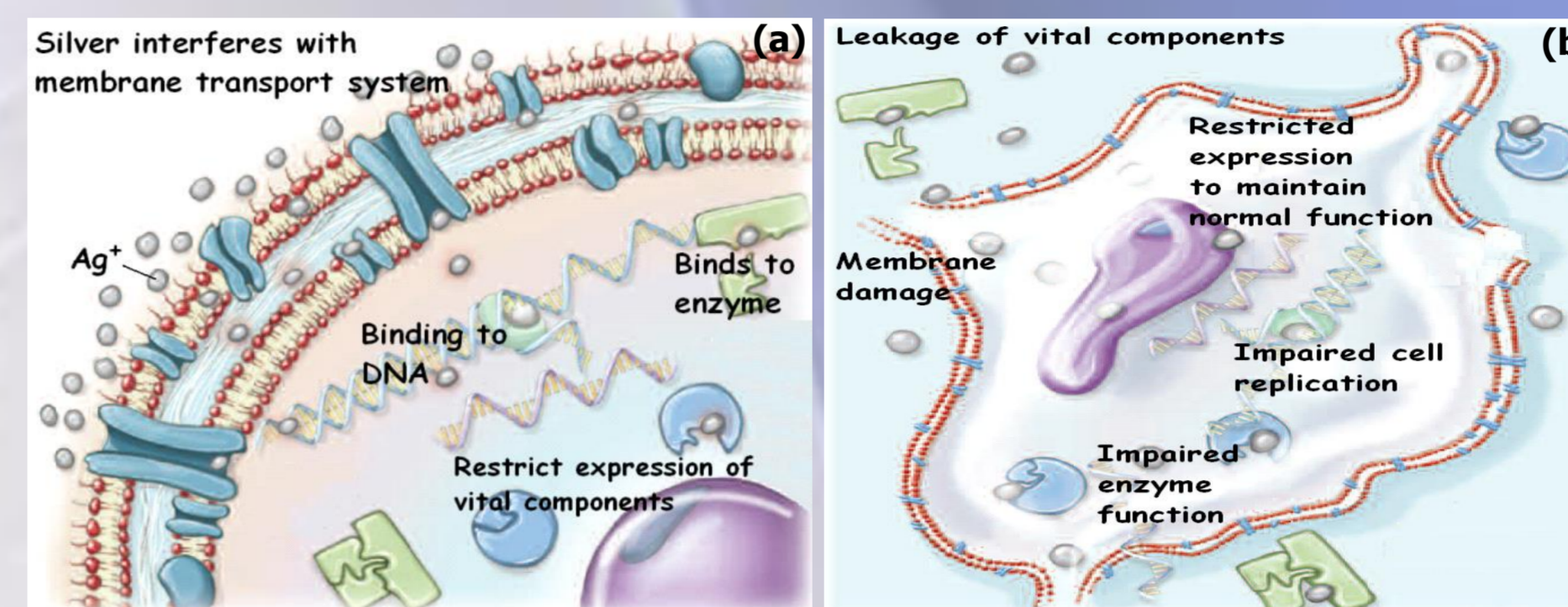


Figure 1. (a) Mode of antimicrobial action of silver (b) Silver damaging bacterial cell organelles.

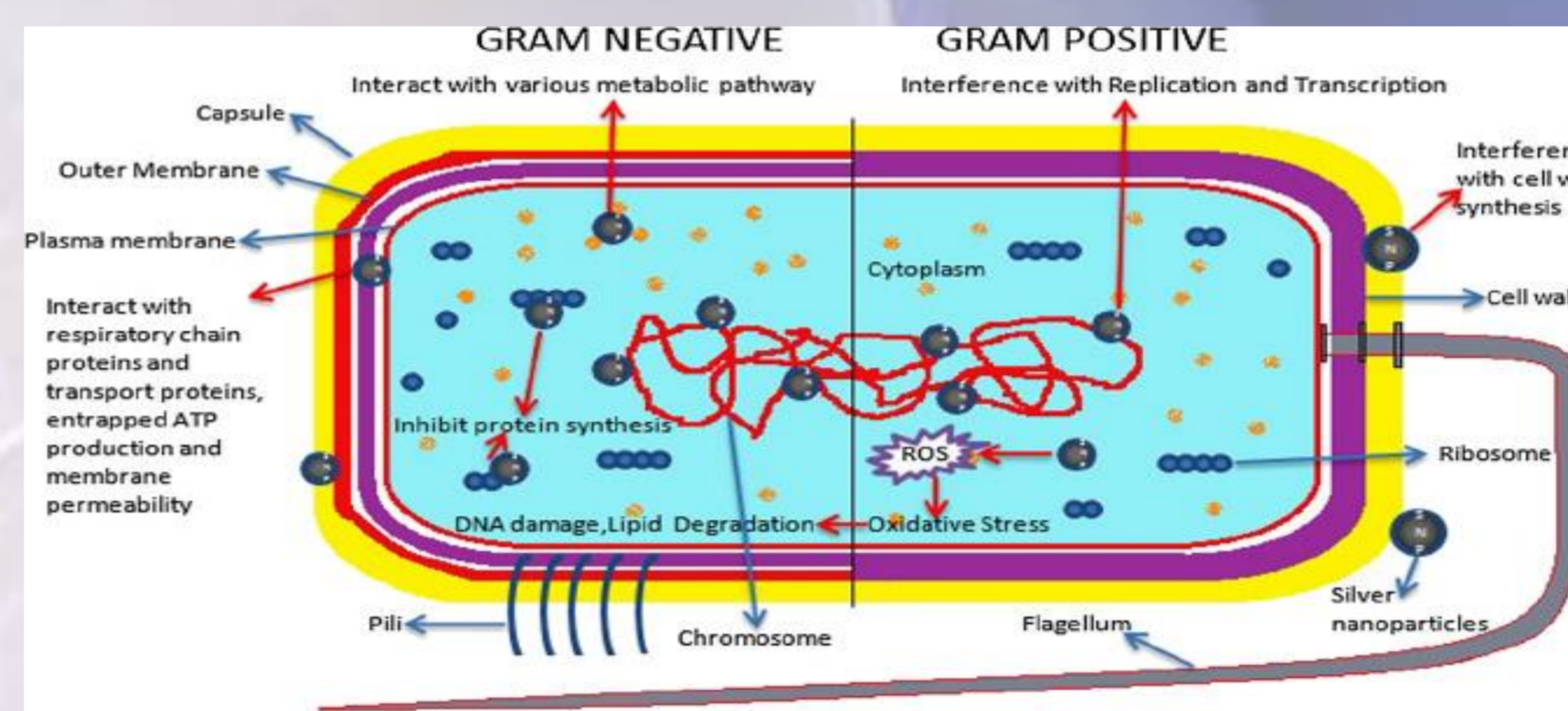


Figure 2. Mode of antimicrobial action of Ag-NP against gram negative and gram positive bacteria [7].

Methodology:

- Synthesis of Ag-NP:** 1mM AgNO₃ was reduced with 5mM NaBH₄ by slow addition and constant stirring.
- Production of BC** (fig.3): *Gluconacetobacter xylinus* ATCC 23770 was selected for biosynthesis of BC. Bacterial colonies inoculated in HS medium and incubated at 30°C.
- Loading of Ag-NP into the BC** (fig.4): BC pellicles were padded dry on the filter paper and loaded with Ag-NP by immersing in colloidal Ag-NP under agitated conditions.
- Testing antimicrobial activity of Ag-NP-loaded BC:**

- Method used:** Disc diffusion assay
- Test Strains:** *S. aureus* (gram +ve) & *P. aeruginosa* (gram -ve)
- Controls:** BC
- Disc size:** 7mm discs of BC and BC-NP
- Total Duration:** After 24 hours ZOI recorded

For your attention: Once purified, bacteria get removed & pure cellulose based hydrogel was ready for Ag-NP loading.

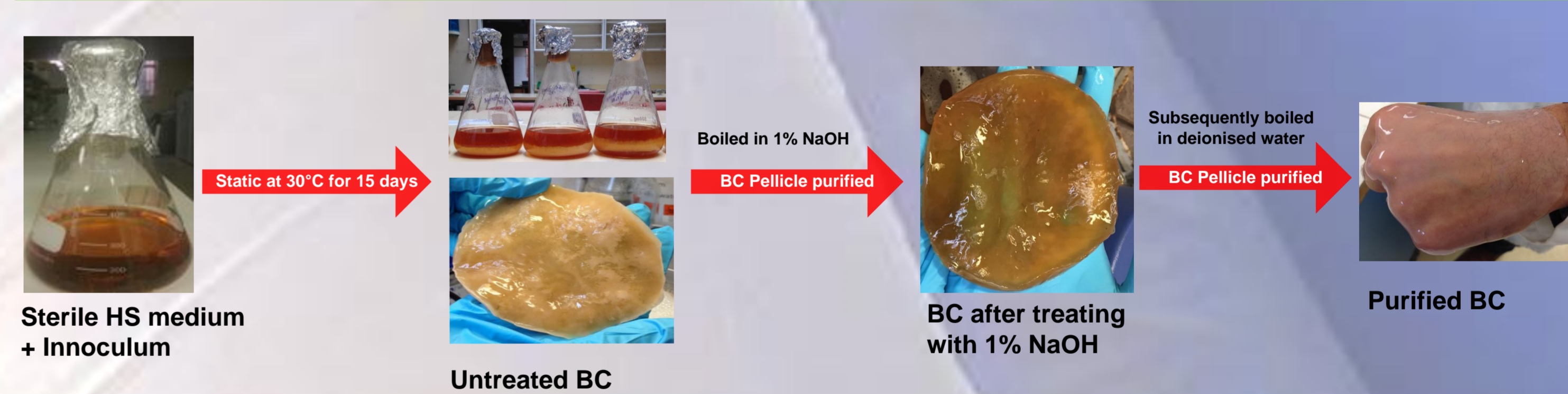


Figure 3. Summary of production of BC.



Figure 4. a) Purified BC before Ag-NP loading b) after Ag-NP loading.

Results and Discussion:

- SEM and EDX results confirmed that BC gets pure after washing (fig. 5). BC has crosslinked fibre network structure (fig. 5b) to accommodate Ag-NP.
- BC does not exhibit anti-microbial activity against *P.aeruginosa* and *S.aureus* (fig. 6a, b). The results confirmed silver in the form of Ag-NP to be the sole candidate for possessing antimicrobial activity against the tested microorganisms.
- It emerged that the Ag-NP loaded BC exhibits higher antimicrobial activity against *P.aeruginosa* compared to *S.aureus* (fig. 6a, b).

For your attention: The greater antimicrobial activity against *P.aeruginosa* compared to *S.aureus* was most likely due to difference in their cell wall structure.

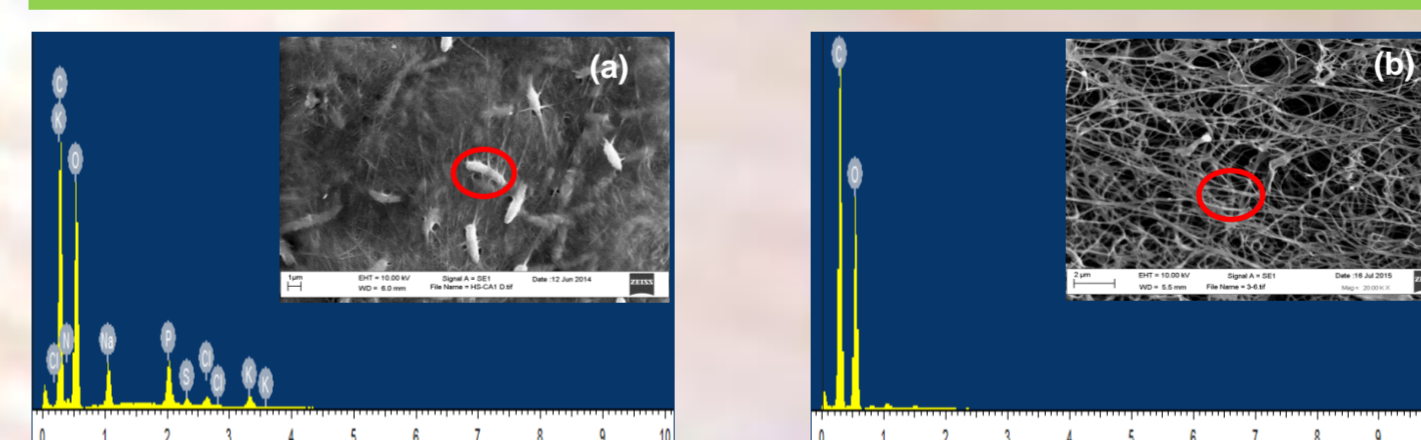


Figure 5. a) SEM and EDX analysis showing BC before purification with bacteria trapped b) SEM and EDX analysis of purified BC showing fibre network structure free from bacteria.

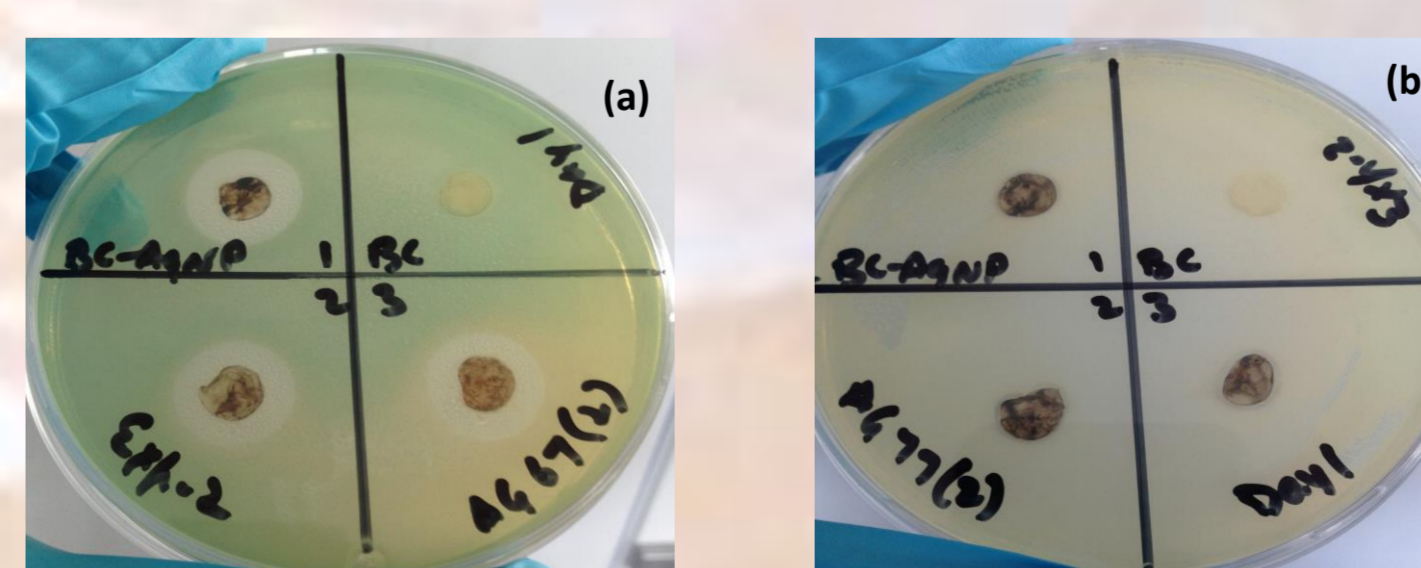


Figure 6. a) & b) Culture plates inoculated with *P.aeruginosa* and *S.aureus* respectively showing ZOI around Ag-NP-loaded BC after 24hours.

Conclusion:

- The hydrophilic and porous nature of BC hydrogel allows impregnation of Ag-NP.
- Ag-NP-loaded BC exhibits antimicrobial activity against both gram negative and gram positive bacteria.
- Results suggest that it is more effective against gram negative, *P.aeruginosa* compared to gram positive, *S.aureus*.

For your attention: The teichoic acid in peptidoglycan layer of Gram +ve bacterial cell wall reduces the effect of Ag⁺.

References:

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Takeaway message:

Ag-NP-loaded BC is a potential candidate for use in wound management due to its antimicrobial activity.