USER GUIDE

GENERATION OF HYDROGELS WITH BOVINE SOLUBLE COLLAGEN

Preparation of hydrogels for 3D cell culture with Bovine Soluble Collagen

Bovine Soluble Collagen can be polymerized to a three-dimensional matrix for 3D cell culture. Choose your application: The following protocols describe how cells can either be embedded inside the hydrogel (protocol A) or seeded on top (protocol B).

Collagen hydrogel for 3D cell culture

GENERAL INTRODUCTION

Viscofan BioEngineering's Bovine Soluble Collagen has a pH of ≅ 3.5 and a collagen concentration of ≅ 5 mg/ml depending on the lot (exact values are given in the Certificate of Analysis). The following protocols describe how to generate neutral hydrogels with customized collagen concentrations with cells either embedded or seeded on top.

Precautions

- Liquids should be kept on ice until hydrogel polymerization is intended. Polymerization is induced by rising pH and temperature. Bovine Soluble Collagen stock solution should be kept on ice when removed from the refrigerator.
- When working with Bovine Soluble Collagen, use appropriate cell culture plastics, media, and reagents as well as aseptic techniques, and ensure adequate conditions for cell growth.
- Due to the high collagen concentration, our Bovine Soluble Collagen has a high viscosity. To avoid pipetting errors, please pipette slowly to allow complete filling and emptying of the tip. Furthermore, dip only the end of the pipette tip into the Bovine Soluble Collagen to avoid transferring any collagen solution that adheres to the outside of the pipette tip.
- For trouble shooting, refer to the section at the end of this protocol or contact our team at sales@bio.viscofan.com.

Required material

- Bovine Soluble Collagen
- Pipettes
- Multiwell plate
- Cell culture medium (e.g., DMEM/Glutamin + 10% FCS)
- Sterile NaOH, 0.1M
- Optionally: analytical pH-paper, range 0-14

INTENDED USE

Bovine Soluble Collagen is intended for research use only. It is neither intended for human nor animal diagnostic, therapeutic use nor any other clinical use.

*Prepare only the amount of mixture needed for the number of hydrogels you can pipette before polymerization begins.

Abbrevations:

$V_{gel\ mixture,\ final}$ [ml]	= volume of final mixture for hydrogels
$V_{per\ gel}$ [ml]	= intended volume per hydrogel
$V_{Bov\ Sol\ Coll}$ [ml]	= required volume of Bovine Soluble Collagen (stock solution)
$C_{Bov\ Sol\ Coll}$ [mg/ml]	= collagen concentration of Bovine Soluble Collagen (stock solution), noted in the Certificate of Analysis
$C_{coll,\ final}$ [mg/ml]	= intended final collagen concentration
$V_{4x\ cell\ suspension}$	= required volume of cell suspension (with 4-fold higher cell concentration than aimed final cell concentration)

A1. Calculate the volumes of the final mixture and of all different components:

Volume of final gel mixture ($V_{gel\ mixture,\ final}$)

$V_{gel\ mixture,\ final} = V_{per\ gel}$ [ml] x number of gels*

Volume of 4x concentrated cell suspension ($V_{4x\ cell\ suspension}$)

Prepare a cell suspension in culture medium, containing a 4-fold cell concentration compared to the aimed final cell concentration (e.g., for a final concentration of $1.5x10^5$ cells/ml in the hydrogel, prepare a cell suspension of $6x10^5$ cells/ml.) The volume needed is calculated by:

$$V_{4x\ cell\ suspension}\ [ml] = \frac{V_{gel\ mixture,\ final}\ [ml]}{4}$$

Volume of Bovine Soluble Collagen (stock solution)($V_{Bov\ Sol\ Coll}$):

Calculate the volume of Bovine Soluble Collagen that is needed for both, the intended number of gels and final collagen concentration:

$$V_{Bov\ Sol\ Coll}[ml] = \frac{V_{gel\ mixture,\ final}\ [ml] \times C_{Coll,\ final}\ [mg/ml]}{C_{Bov\ Sol\ Coll}\ [mg/ml]}$$

Volume of 0,1 M NaOH:

The volume of 0,1 M NaOH to raise the pH of the mixture to neutrality is dependend on the volume of Bovine Soluble Collagen used:

$$V_{NaOH,\ 01,\ M}\ [ml] = \frac{V_{Bov\ Sol\ Coll}\ [ml]}{6}$$

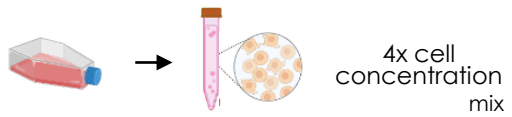
Volume of cell culture medium (V_{medium})

$$V_{medium}\ [ml] = V_{gel\ mixture,\ final}\ [ml] - V_{Bov\ Sol\ Coll}\ [ml] - V_{NaOH,\ 01,\ M}\ [ml] - V_{4x\ cell\ suspension}\ [ml]$$

A2. Mix the calculated volumes under sterile conditions as follows:

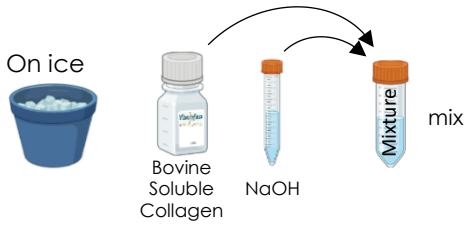
STEP 1

Prepare a cell suspension in cell culture medium with a 4-fold higher cell concentration than the desired final cell concentration in the hydrogel ($V_{4x\ cell\ suspension}$). Keep at room temperature until adding it to the collagen mixture in step 4.



STEP 2

Prepare on ice in a second vial ("mixture"): Ensure homogeneity of the collagen stock solution by pipetting it up and down several times, then pipette the calculated $V_{Bov\ Sol\ Coll}$ into the vial. Subsequently raise the pH by adding V_{NaOH} and mix briefly but thoroughly.

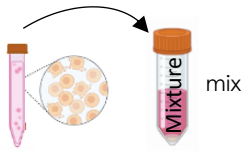


STEP 3

Quickly add V_{medium} to the mixture and mix thoroughly.

STEP 4

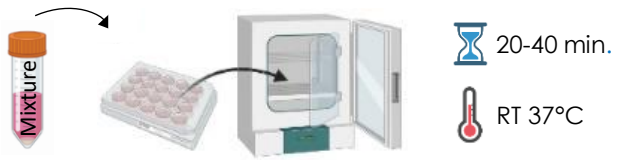
Quickly mix gently but thoroughly with $V_{4x\ cell\ suspension}$ from step 1.
Optionally, to check for neutral pH, pipette a few drops on pH-paper. (A slightly acidic pH will turn neutral after incubation in the CO_2-incubator.)



STEP 5

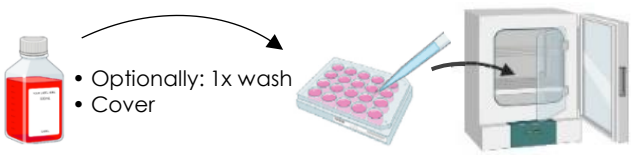
Quickly pipette $V_{per\ well}$ of the mixture into the wells of a multiwell plate avoiding bubbles.

Allow the hydrogels to polymerize completely at 37°C in approximately 20 - 40 min. without moving the well plate during this time.



STEP 6

Optionally, wash the hydrogels once gently with medium. Finally, cover the hydrogels with medium and incubate at proper culture conditions (e.g., 37°C and 5% CO_2).



Bovine Soluble Collagen
Order-number: 500060635

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$C_{Bov\ Sol\ Coll}$ [mg/ml]	= collagen concentration of Bovine Soluble Collagen (stock solution), noted in the Certificate of Analysis
$C_{Coll,\ final}$ [mg/ml]	= intended final collagen concentration

B1. Calculate the volumes of the final mixture and of all different components:

Volume of final gel mixture ($V_{gel\ mixture,\ final}$)

$V_{gel\ mixture,\ final} = V_{per\ gel}$ [ml] x number of gels*

Volume of bovine soluble collagen (stock solution)($V_{Bov\ Sol\ Coll}$):

Calculate the volume of Bovine Soluble Collagen that is needed for both, the intended number of gels and final collagen concentration:

$$V_{Bov\ Sol\ Coll}\ [ml] = \frac{V_{gel\ mixture,\ final}\ [ml] \times C_{Coll,\ final}\ [mg/ml]}{C_{Bov\ Sol\ Coll}\ [mg/ml]}$$

Volume of 0,1 M NaOH:

The volume of 0,1 M NaOH to raise the pH of the mixture to neutrality is dependend on the volume of Bovine Soluble Collagen used:

$$V_{NaOH,\ 01,\ M}\ [ml] = \frac{V_{Bov\ Sol\ Coll}\ [ml]}{6}$$

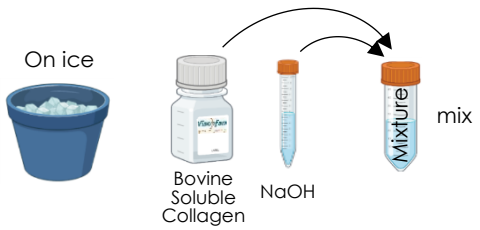
Volume of cell culture medium (V_{medium})

V_{medium} [ml] = $V_{gel\ mixture,\ final}$ [ml] – $V_{Bov\ Sol\ Coll}$ [ml] – $V_{NaOH,\ 01,\ M}$ [ml]

B2. Mix the calculated volumes under sterile conditions as follows:

STEP 1

On ice: ensure a homogeneous stock collagen solution by pipetting it up and down several times, then pipette the calculated $V_{Bov\ Sol\ Coll}$ into a vial. Subsequently add V_{NaOH} to raise the pH and mix briefly but thoroughly.

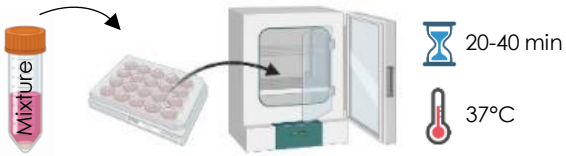


STEP 2

Quickly add V_{medium} to the mixture and mix thoroughly.
Optionally, to check for neutral pH, pipette a few drops on pH-paper. (A slightly acidic pH will turn neutral after incubation in the CO_2-incubator.)

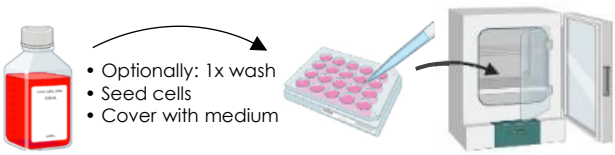
STEP 3

Quickly pipette $V_{per\ well}$ of the mixture into the wells of a multiwell plate avoiding bubbles. Allow the hydrogels to polymerize completely at 37°C in approximately 20 - 40 min. without moving the well plate during this time.



STEP 4

Optionally, wash the hydrogels once gently with medium.
The hydrogels are now ready for cell seeding. Cover the hydrogels with medium until cell seeding to prevent them from drying. After cell seeding cover the hydrogels again with medium and incubate them at appropriate culture conditions.



Tool-symbols generated with BioRender

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ISSUE	TIPS
1. Hydrogel fails to polymerize	• Do not move the well plate during the hydrogel polymerization step (step 5 in protocol A and step 3 in protocol B, respectively). • Check whether the final collagen concentration is high enough to enable polymerization to a hydrogel (generally at least 0,5 mg/ml) • Please check whether the pH of your final mixture is neutral to slightly acidic and adjust V_{NaOH} if necessary (see also issue 5.).
2. Hydrogel does not polymerize homogenously	• Especially when aiming at high collagen concentrations, be sure to work fast after addition of NaOH (steps 2-5 in protocol A and steps 1-3 in protocol B). • Keep liquids on ice until hydrogel polymerization is intended (= by raising pH and temperature). • Do not move the well plate during the hydrogel polymerization step (step 5 in protocol A and step 3 in protocol B, respectively).
3. Hydrogels appear yellowish instead of red	• Incubation with CO_2 influences the pH level of the cell culture medium and the hydrogels. Especially at high collagen concentrations hydrogels might be slightly acidic (=yellowish) shortly after polymerization but may change to neutral (red) within 1-2 hours during CO_2-incubation (with phenol red as a pH-indicator). If in doubt whether a neutral pH has been reached, or if your cells are very sensitive to even slight and brief acidic conditions, follow the tips in issue 5.
4. Cells die or fail to grow	• Ensure a neutral pH of the final mixture and adjust if necessary (see issue 5.) • Check whether the chosen hydrogel density and/or cell seeding density is suitable for your cell type. • Check whether enough cell culture medium has been added to or on top of the hydrogel to feed the cells. • Try a gentler mixing method in protocol A, step 4.
5. Ensuring, checking and adjusting pH of the hydrogel	• The protocols are designed to generate hydrogels with neutral pH. However, e.g., high collagen end concentrations may need additional NaOH. If in doubt, whether neutral pH has been achieved, perform a pre-test with a pH-meter and adjust V_{NaOH}, if necessary. • Alternatively, as a quick check, pipette a few drops of the final mixture on pH paper before gelation starts. It should be neutral to slightly acidic. • To avoid pipetting errors caused by the slow-flowing properties of Bovine Soluble Collagen, pipette slowly and consider using wide-bore tips. Dip only the end of the tip into the collagen solution and avoid transferring any liquid that adheres to the outside of the pipette tip.

Contact us
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Use collagen scaffolds to improve performance of your 2D & 3D cultures!

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ISSUE	TIPS
6. During polymerization embedded cells sink to the bottom below the hydrogel	• Before generating a cell-embedded hydrogel, generate a thin hydrogel without cells at the bottom of the well using protocol B. Wait until polymerization is finished. Then mix and pour your mixture containing embedded cells on top of the first hydrogel using protocol A. • Consider working with a higher collagen concentration.

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