Gelatin promotes rapid restoration of the blood brain barrier after acute brain injury

Lucas S. Kumosa, Valdemar Zetterberg, Jens Schouenborg

Lund University

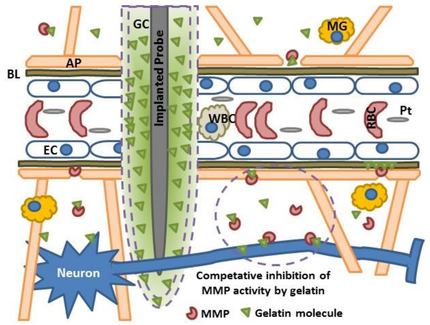
Neuronano Research Center

Purpose

Gelatin coating of brain intrusions is known to provide considerable benefits in terms of reduced inflammatory sequalae and long-term neuro-protective affects.

This project aims to elucidate the mechanisms for gelatin's protective role in brain injury.

Hypothesis



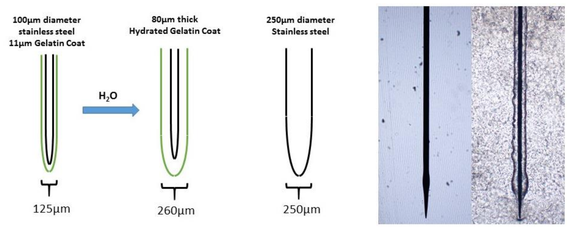
MG – microglia
WBC – white blood cell
RBC – red blood cell
GC – gelatin coating

AP – Astrocyte process
BL – basal lamina
EC – endothelial cell
Pt - platelet

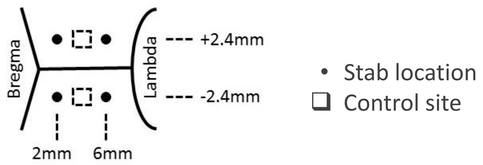
Gelatin as a common substrate for gelatinases and collagenases (two families of matrix metalloproteinases (MMPs)) competitively inhibits MMP degradation of the basal lamina, limiting post-insertion bleeding and allowing the blood-brain barrier (BBB) to heal rapidly.

Needles and Stab Injury

Transient insertion of stainless steel needles into rat cortex cerebri with or without gelatin coating.



Gelatin dimensions: 11 ± 3 µm dry, 80 ± 19 µm hydrated.



Female Sprague-Dawley rats (258 ± 14 grams, n = 55) were used (Animal Ethics Committee of Malmö/Lund, Sweden, under permits M338-12 and M76-16).

Serial Immunohistochemistry

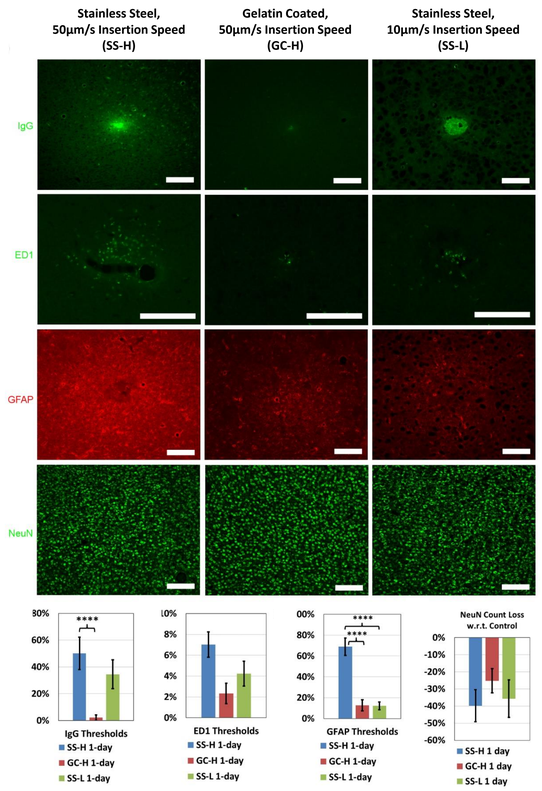
Cellular and molecular markers were studied at acute (<2 hours, 1, 3 days), intermediate (1-2 weeks) and long-term time points (6 weeks) after insertion.

Staining: IgG – BBB leakage, ED1 – activated microglia, Iba1 – all microglia, GFAP – astrocytes, NeuN – neurons, MMP-2 and MMP-9 – gelatinases.

Quantitation: Image processing and quantification performed using Matlab algorithms specifically written for this application.

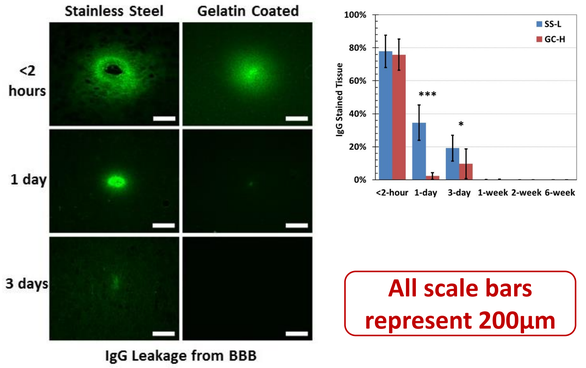
Statistics: Mann-Whitney test, p-values are labeled as **** (<0.001), *** (<0.005), ** (<0.01), or * (<0.05), all error bars represent standard error of the mean (SEM).

Acute (1-day) Response



There is almost no detectable IgG leakage in the gelatin coated stab site compared to stainless steel needles. GFAP expression is significantly stronger in the faster stainless steel induced stabs than in both slower induced stabs and gelatin induced stabs, which behave identically.

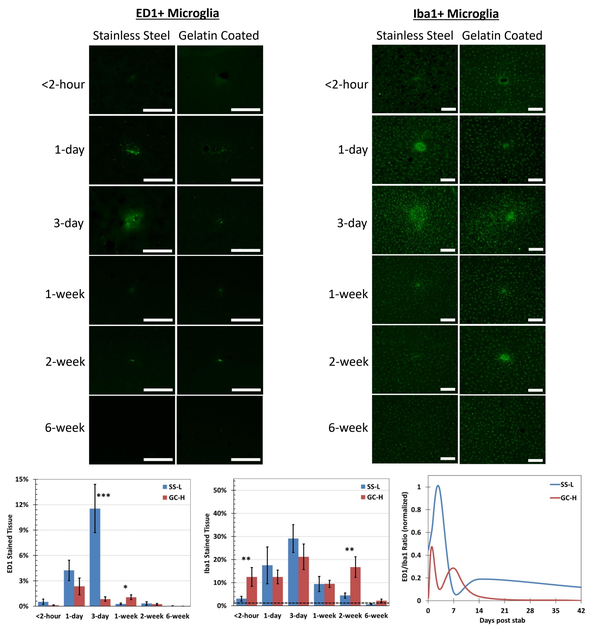
BBB Leakage



All scale bars represent 200µm

IgG leakage from the neurovasculature adjacent to the wound site is resolved after 3 days in the case of stainless steel needles and brought to small levels within 1 day for the gelatin coated stabs.

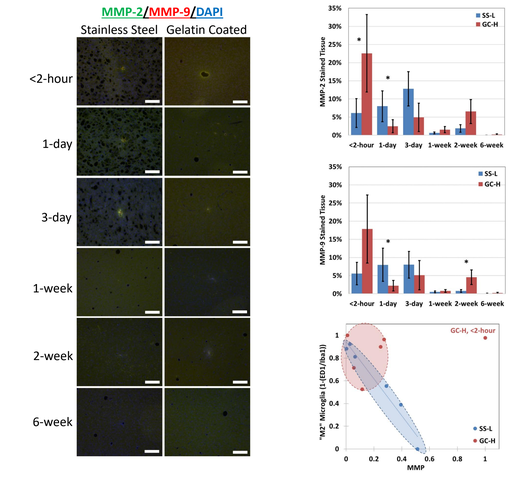
Microglial Modulation



Iba1+/ED1+ microglia ("M1") are reduced by gelatin. By contrast, we found a rapid increase followed by a sustained level of Iba1+/ED1- microglia ("M2") in the gelatin group lasting over 2 weeks.

Gelatin induces a preferential "anti-inflammatory, neuroprotective" population of microglia and limits the "pro-inflammatory, digestive" phenotype.

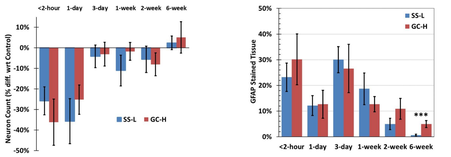
Gelatinase Dynamics



Much stronger initial release of MMPs in the gelatin group as compared to steel controls suggests tissues recognize gelatin and release gelatinases to break it down.

MMP activity was found to be inversely correlated with "M2" microglial presence; gelatin inhibits MMPs.

Neurons and Astrocytes



NeuN staining is transiently reduced after injury then returns to normal after 3 days, irrespective of gelatin. The astrocytic response caused by both stainless steel and gelatin coated needles is nearly identical.

Ubiquitous loss of neurons around chronic brain implants cannot be explained by the implantation procedure itself.

Conclusions

1. Gelatin shortens acute BBB leakiness and mitigates early inflammatory sequalae.
2. Competitive inhibition of gelatinases in acute phase may protect the BBB from MMP-induced basal lamina degradation and provide an amino acid-rich environment for repair.
3. Careful insertion of rigid structures does not by itself have much long-term impact on neurons, astrocytes.

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